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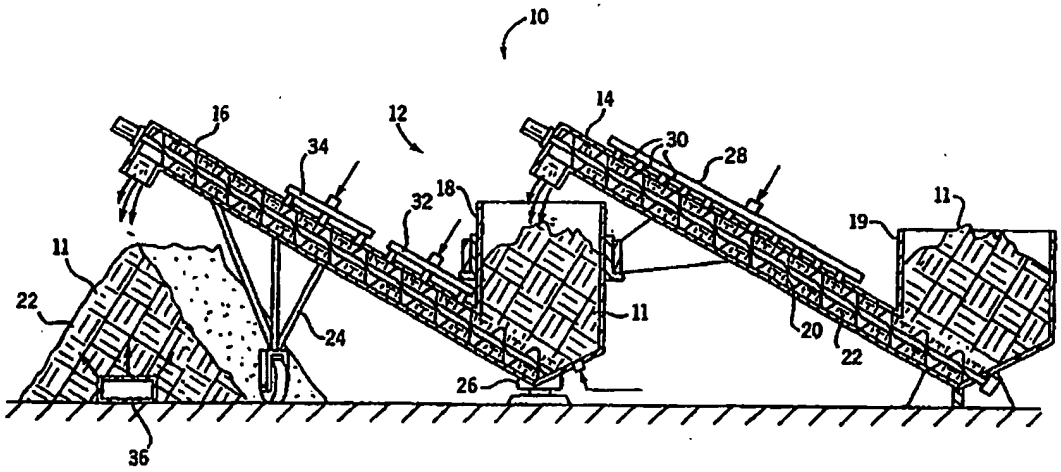

(57) Abstract

A biopulping process is provided which is capable of scale up to commercial quantities. The process utilizes a biosuppression step in which contaminating microorganisms which interfere with the colonization and propagation of lignin–degrading or modifying organism, are physiologically disabled. The process involves inoculating (34) steam–treated wood chips (11) with a fungus, followed by incubation for a period of time necessary to achieve the desired degree of lignin degradation or modification. The invention also provides an apparatus for treating and inoculating (34) in continuous mode a large commercial quantity of wood chips (11). Use of continuous chip conveyance (12) ensures process control in a small, manageable environment thereby promoting uniformity of contaminant biosuppression, and colonization and even propagation of the fungus on the chip surface.

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METHOD AND APPARATUS FOR COMMERCIAL SCALE BIOPULPING

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FIELD OF THE INVENTION

This invention relates to the field of paper manufacture and more particularly, to controlled biological degradation on a commercial scale of the lignin component of wood by white rot fungi. The methods also apply to other useful biological treatments of wood in the pulping process.

BACKGROUND OF THE INVENTION

When paper is manufactured from wood, the wood is first converted into an aqueous suspension called pulp. The purpose of pulping is to separate the wood fibers from a complex solid matrix. The most abundant component of the native wood matrix is cellulose, a polysaccharide that is desired for paper production. The second most abundant component of native wood is lignin, a complex polymer made of aromatic units. Lignin is undesirable in paper manufacturing because it forms complexes with cellulose ("lignocellulosics") in the native wood, making the separation of the desired cellulose wood fibers more difficult during the pulping process.

Pulp can be produced by mechanical methods, in which the wood is ground or abraded in water. Heat may be added in a process called thermo-mechanical pulping. Mechanical and thermo-mechanical pulping, however, require high energy input to break up the lignocellulosic complexes in order to free the cellulose wood fibers from the native wood matrix. Mechanical pulping also produces low strength pulps that are unsuitable for producing products with high

strength requirements. Other pulping methods use chemicals with or without subsequent mechanical treatment. The chemical treatments degrade or modify the lignin and may increase the strength properties of paper resulting after subsequent mechanical pulping. However, these chemical or chemi-mechanical pulping methods produce waste streams which must be treated, may have no effect on the energy requirements in mechanical pulping, and produce pulps with much lower yield.

In biopulping, the wood is treated with an organism that breaks down the native wood matrix such that subsequent pulping steps require less energy or result in products of higher strength characteristics. Biopulping research has focused exclusively on the white-rot fungi, so named because of their preferential degradation or modification of lignin, resulting in a pale colored wood. Work with the white-rot fungi before 1985 established that treatment of wood chips with several of these fungi may result in energy savings in subsequent mechanical pulping steps and may increase the strength of the paper which was then produced, as disclosed in U.S. Patent No. 3,962,033. Fungi which showed these encouraging results included isolates from the genera *Rigidoporus*, *Phanerochaete* (also called *Sporotrichum*), *Trametes*, *Polyporus*, *Peniophora*, and *Coriolus*.

Extensive research into white-rot fungi biopulping has been conducted by a consortium ("the biopulping consortium") formed in 1987 by the University of Wisconsin - Madison, the USDA Forest Service, Forest Products Laboratory, and several industrial partners. Early consortium experiments established standardized laboratory-scale methods to screen various isolates of white-rot fungi for their ability to colonize wood chips. Many of these isolates exhibited that ability, including isolates from the genera *Phlebia*, *Dichomitus*, *Phanerochaete*, *Poria*, *Hypodontia*, and *Ceriporiopsis*.

One species, *Ceriporiopsis subvermispora*, was selected for further study because it appeared superior to other species tested, as disclosed in U.S. Patent No. 5,055,159, incorporated herein by reference.

Other improvements arising from the biopulping consortium have been disclosed. U.S. Patent No. 5,460,697 teaches a method of treating the wood chips with sulfite salts, which allows *C. subvermispora* to grow on the wood chips, but prevents propagation of contaminating microorganisms. Another pending U.S. Patent Application, Serial No. 08/289,429, incorporated herein by reference, discloses a method for enhancing biopulping efficacy through the reduction of inoculum of *C. subvermispora* required to inoculate the wood chips. By adding certain nutrient adjuvants such as corn steep liquor, molasses, or yeast extract to the inoculum, the amount of inoculum required to effectively inoculate the wood chips can be reduced by over 100-fold. Thus, the amount of inoculum required for commercial scale biopulping will be reduced to a practical level.

The work of the biopulping consortium and others has shown that there are many species which might serve as an effective biopulping fungus. *C. subvermispora* was chosen as the best species in tests of a group of seven species, but several of the groups performed nearly as well in those evaluations. Indeed, pending U.S. Patent Application Serial No. 08/682,813 discloses that one of those fungi, *Phlebia subserialis*, proved to have the unexpected property of growth almost exclusively internal to the wood chips, whereas *C. subvermispora* produces extensive growth on the outside of the wood chips.

The internal growth characteristics of *P. subserialis* could be an advantage over *C. subvermispora* in a large scale commercial biopulping process. The external wood chip growth of *C. subvermispora* in a large scale biopulping process may cause a restriction

in the air flow through an incubated wood chip pile. The air flow removes the heat generated by the growing fungus. By contrast, the internal growth of *P. subserialis* may not cause a similar restriction with the ventilation required in a large scale process.

P. subserialis also grows at a higher temperature than *C. subvermispora*. Therefore, a biopulping incubation with *P. subserialis* may require less ventilation to remove heat than *C. subvermispora*. *P. subserialis* also proved to be comparable to *C. subvermispora* in energy savings produced in subsequent pulping, and in improved strength characteristics in the resulting paper product. Thus, although *P. subserialis* was somewhat inferior to *C. subvermispora* in early comparisons, it proved to be similar to *C. subvermispora* when compared later, and had a growth characteristics that may prove advantageous to *C. subvermispora* in a commercial biopulping process. This shows that many white-rot fungi are suitable for biopulping and may be as good or better than *C. subvermispora*.

Previously, there was little information available regarding the characteristics of a suitable method for commercial scale (40 tons or greater) biopulping. All previous biopulping work was performed on a small scale, using less than 10 pounds of wood chips.

SUMMARY OF THE INVENTION

In the biopulping field, demonstration that selected microorganisms could aid the pulping process by digesting away the lignin portion of wood, thereby releasing the cellulosic fibers, has focused on laboratory scale identification of the organisms and their properties. Several recent advances have set the stage for commercial scale biopulping processes, including identification and pure culture propagation techniques of specific white rot fungi which degrade or modify lignin but do not attack cellulose, and

nutritive strategies for making inoculation of wood cost-feasible on an industrial scale. A number of serious impediments to commercialization remained. These include the absence of a precision-controlled process capable of handling very large volumes of wood, and the absence of a process not requiring sterilization of the wood to obtain selective growth and metabolic action of a specific organism.

The present invention addresses and solves these latter impediments, and makes possible for the first time a practical commercial scale biopulping process. Since the primary purpose of a biopulping step is to reduce the costs of mechanical fiber separation of ordinary wood chips, biopulping must yield a net return in paper quality, energy savings, and throughput increases to be practical. Accordingly, it is an object of the present invention to combine the control precision of a small laboratory batch process in a bioreactor with the requirement for handling a large volume of wood in commercial scale.

Another object is to attain biosuppression of the naturally occurring contaminating flora in favor of the often more fastidious biopulping organism, so that lignin degradation or modification is a principal metabolic activity during the course of biopulping. A still further object is to devise an incubation strategy that permits the biopulping process to continue for the desired time period without disruption by accumulation of heat inhibitory to the biopulping organisms in the chip mass.

According to the present invention, commercial scale biopulping involving wood chip volumes from several hundred kilograms to hundreds of tons comprises, first, heating the chip surfaces to a temperature of at least about 50°C to 100°C by applying steam to the surfaces thereof ambiently, or under pressure from at least 110 psi to 150 psi, for a time sufficient to disable or biosuppress the contaminating

population of native, naturally occurring microbial flora, without complete sterilization of the chips, at least not beyond the surface of the chips or portions thereof. In the second step, the chips are cooled to a temperature physiologically suitable for inoculation of a lignin-degrading or modifying species of white rot fungus, or other organism which selectively degrades or modifies lignin and does not degrade cellulose, followed by inoculating the surfaces of the chips with an inoculum of fungus suspended in a growth promoting nutrient solution so that no substantial portion of the surfaces is uninoculated. Finally, the chips are incubated under conditions of controlled temperature compatible with the propagation of the fungus until the lignin contained therein is degraded or modified to the extent desired for mechanical pulping.

In an alternative embodiment to holding or maintaining the chips in a set permissive temperature condition, it is possible to carry out the incubation where the natural evolution of heat from biometabolism results in elevation in temperature of the chip mass to reach a temperature higher than optimum for the organism, and yet still obtain a good biopulped product. This cannot be done by an abrupt temperature shift in a bioreactor, but only by natural conditioning of the fungi over the course of the biopulping cycle.

In another aspect, the invention provides a method of continuously treating wood chips for biopulping or other biotreatment. The method comprises the steps of first feeding the wood chips into a conveyor, then conveying the wood chips over a distance on the conveyor and agitating the wood chips during the conveying step such that the wood chips are moved sufficiently to permit access to a substantial portion of the surfaces of the wood chips and thereby performing various treatments while the wood chips are being conveyed, and finally collecting the wood chips so treated. Those treatments may include heating the

wood chips during the conveying step by passing the wood chips through an amount of steam, cooling the chips during the conveying step to a temperature physiologically suitable for inoculating fungi, and dispersing an amount of inoculant over a substantial portion of the surfaces of the wood chips, the inoculant comprising an inoculating fungi and a nutrient adjuvant.

In yet another aspect, the invention provides an apparatus for continuously treating wood chips for biopulping or other biotreatment. The apparatus comprises an input source, such as a feeder, for feeding wood chips into a conveyor and a conveyor interconnected to the input source. The conveyor includes a means for agitating the wood chips such that a substantial portion of the surfaces of the wood chips is exposed to treatment. The apparatus further comprises a decontaminator having an amount of decontaminant and being capable of dispersing the decontaminant throughout the wood chips such that naturally occurring organisms are at least partially disabled or their growth is suppressed, and an inoculator having an amount of fungal inoculum and being capable of dispersing the inoculum through the wood chips such that the fungal inoculum may propagate.

In a preferred embodiment, the decontaminator consists of a heater, such as a steam heater, and a cooler. At least one of either the heater, the cooler, or the inoculator may be mounted to the conveyor such that the heating, cooling, or inoculating may be performed continuously on wood chips being conveyed. Further, the conveyor may comprise a first conveyor portion and a second conveyor portion operatively connected by means of a surge tank. The heater may then be mounted to the first conveyor portion, and the cooler and inoculator may be mounted to the second conveyor portion. In such a conveyor, the action of the heater, the cooler, and the inoculator may be more

separately and precisely controlled. The conveyor may comprise a screw conveyor, such as an auger enclosed within a housing. In a conveyor of such a configuration, the agitating means is the conveyor itself. However, different agitating means may be employed that may be mounted to the conveyor or included within the conveyor. Finally, the conveyor may be movably mounted such that wood chips expelled from the conveyor are spread over a distance.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a plan view of an apparatus for treating and inoculating wood chips, incorporating screw conveyance of chips through a steam treating zone and an inoculating zone.

Fig. 2 is a plan view of a treating and inoculating apparatus operating on an axis of rotation, for deposition of treated chips in an arc.

Fig. 3 is a schematic showing various functional zones of a chip pile.

Fig. 4 is a schematic showing the position of temperature sensors within a typical chip pile.

Fig. 5 is a graph of temperature profiles over a two week incubation.

Fig. 6 is a schematic of a bioreactor showing the relation of major components.

Figs. 7-9 are temperature profiles for various experiments.

Fig. 10 is a temperature profile for a typical chip silo run.

Fig. 11 is a graph showing the compression of treated wood chips and untreated chips as a function of applied load.

Fig. 12 is a schematic of a 60 kg chip pile.

Fig. 13 is a temperature profile for a two week unventilated incubation.

Fig. 14 is a schematic of a free-air silo.

Fig. 15 is a graph showing temperatures during biopulping in a free-air silo.

Fig. 16 is a graph showing temperatures during biopulping in a free-air silo.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Scaling up biopulping processes to commercial quantities is found to present special problems not observed in laboratory trials. In the laboratory, or even the pilot plant, the various steps in preparing the wood chips, inoculating them with fungal solutions, and the subsequent 1 to 4 week incubation can all be carried out using manual procedures and self-contained laboratory equipment. For example, mixing the inoculum presents no particular problem because all that is involved is stirring in the nutrient-containing fungal inoculum. Conveniently the biopulping reaction incubation can then be routinely carried out in a rotating drum or air-lift bioreactor as described in pending U.S. Patent Application No. 08/289,429, hereby incorporated by reference. U.S. Patent No. 5,055,159, hereby incorporated by reference, further discloses a laboratory scale bioreactor constructed of simple laboratory implements and glassware.

Under such laboratory conditions, absolute sterility of both the biopulping mass and even the

influent air can be maintained and temperature is easily controlled. It is also straightforward to monitor the process by removing representative samples. The enclosed bioreactor ensures process uniformity throughout the vessel. Prior to Applicants' invention herein, it was unknown whether biopulping could be applied on a commercial level, because it is both technically and economically infeasible to design a system having the process control available in the laboratory.

In the present process, Applicants' have discovered that effective biopulping can be carried out under nonsterile conditions in which naturally occurring flora are present and viable. Wood chips are exposed to live steam resulting in elevating their surface temperature to about 90° to 100°C, as measured immediately after steam treatment. The exposure time is a function of the temperature of the superheated vapor and also the inlet pressure. While 101° to 108°C influent steam at 15 to 75 in line psi for exposure times of 3 to 50 seconds is adequate, the optimum values are best determined in a few empirical process runs for the particular type and configuration of equipment, as hereinafter described in more detail.

The chamber in which steam treatment takes place should not be too tightly packed. Open space of about under 10% to over 65% of the volume capacity is sufficient to allow penetration of steam to all chip surfaces provided that the chips can be mechanically turned or agitated to prevent impeded exposure to steam at touching surfaces. For example, in the screw conveyor used in a preferred embodiment of the invention, the open space above the chips in the conveyor was found to be approximately 57% to 69%. (In addition, the void space between the chips amounted to approximately 61%. Therefore, the total void space in the conveyor amounted to approximately 83% (large chips) to 88% (small chips).) Uniformity of steam

treatment is very important, as the naturally occurring flora must be uniformly disabled or biosuppressed physiologically to avoid spots of overgrowth by contaminants during the subsequent incubation step.

A particularly efficient method of steam treatment is by injecting steam into a continuous flow screw or auger bearing the chips at about 30% to 45% spacial density as discussed above. It was found that exposure time of chips adequate for the present process could be only 40 seconds compared to 5-10 minutes in a quiescent batch mode. Steam was released at moderate pressure and applied ambiently without pressurizing the vessel.

Another surprising discovery was finding that the chips do not require complete sterilization. A number of species of contaminating organisms can readily be isolated from moistened wood chips including *Aspergillis spp.*, *Colletotrichum spp.*, *Trichoderma spp.*, *Gliocladium spp.*, *Ophiostoma spp.*, *Penicillium spp.*, *Ceratocystis spp.* , *Nectria spp.*, *Cytospora spp.*, and *Alternaria spp.* Many of these are more physiologically robust and faster growing than the inoculating lignin-degrading or modifying fungi of choice. Growth of these organisms is also enhanced in many instances by the nutrient adjuvants contained in the fungal inoculum.

Further surprising aspect of nonsterile incubation is that the process control and quality of paper obtained from the present process are superior to that seen in the situation where the chips are actually sterilized. The physiological and ecological balance amongst the several contaminating organisms and the primary fungus may have advantages in the biochemical process of lignin degradation or modification over the situation in which the inoculated fungus has unrestricted metabolic access to the chips. When environmental circumstances become compromised or even adverse to the white rot fungi, the process may become unstable.

Once the indigenous, undesirable microbes are disabled or suppressed by steam treatment, the less robust and more fastidious white-rot fungi in the inoculum are able to remain dominant over extended periods. The disabled organisms are still viable and capable of becoming dominant, as shown by biopulping runs in which the treatment temperature was inadvertently allowed to rise only to suboptimal levels. In those instances the runs were ruined by overgrowth of the contaminating fungi. Clearly a highly delicate but controllable process balance must be maintained, but it is unclear scientifically what competitive factors are at work to maintain the desired biological balance over extended incubations. Reducing exposure to steam to a minimum without sterilization also has favorable implications for process costs. The low exposure time conductive to a continuous treatment means that high volume treatment required in any commercial scale process is attainable in the present invention.

In the next step of the process, the chips must be cooled sufficiently to permit inoculation of the biopulping fungi without killing or disabling them. Many of the useful species may actually be more sensitive to elevated temperatures than their naturally occurring flora counterparts. Chips steam treated on a continuously moving path are passed through heat transfer means which cool the chips to an appropriate temperature for inoculation. Applicants have found that the most cost effective and simplest method is to place an in-line air blower manifold directly in the conveyance path, and adjust the air flow to a rate that will cool the passing chips adequately.

It has been determined empirically that chips cooled to about 40°-45°C. and as high as 50°C. are cool enough not to heat shock the fungi contained in the inoculum. The highest temperature tolerated by biopulping organisms may vary from species to species

so that some empirical tests may be necessary to determine a physiologically suitable temperature for inoculation of that species. Cooling only to the highest physiologically suitable temperature minimizes the cooling time and speeds the process, and reduces the energy consumed.

Inoculation of the biopulping fungi is preferably carried out in-line, and applied as a liquid spray to the passing wood chips. As in the steam treatment, the working action of agitated conveyor or auger allows inoculum to be uniformly adsorbed onto the chip surfaces by tumbling and churning during rotary or other agitated conveyance. It is important that the inoculum be applied substantially thoroughly and uniformly to the chip surfaces. If the biopulping fungi are to maintain dominance over other flora, the contaminating flora should not be given a sufficient opportunity to reestablish themselves in local areas of the chip surfaces where coverage of inoculum is uneven.

In the laboratory, subsequent incubation of the chips can readily occur in the controlled environment of a bioreactor. Commercially, bioreactors having the control features of laboratory models are impractical from an economic standpoint. Further, maintaining tightly controlled process conditions is expensive and time consuming on commercial sized batches. A typical pulping operation may process chips in batches of up to 200 to 500 tons. Commercial scale biopulping involves quantities as low as 40 tons, although some of the technical problems that arise upon scaling to commercial quantities begin to become evident even at 4 tons, although incubations can be carried out in a closed container at that quantity.

A very important by-product of the commercial scale process not presenting any problem under laboratory conditions is heat generation. The enzymatic breakdown or modification of lignin by fungi is an exothermic reaction, so that when a large mass of

chips is undergoing delignification, a substantial concentration of heat ensues. As the surface area of the mass of chips diminishes relative to the total mass, the problem intensifies since wood itself is an excellent heat insulator. The most practical way to dissipate heat in the chips to prevent the temperature from exceeding the level at which the biopulping fungi are killed, and the contaminants begin to overgrow the fungi, is by forcing air through the chips.

Applicants have found that the temperature of chip piles can be adequately controlled and maintained at levels biocompatible with the continued propagation and dominance of the fungus by loading the chips onto an air pervious frame defining a plurality of ducts through which forced air is passed. It has been empirically determined that the humidity of the air should be in a range from at least 30% up to over 95% relative humidity, preferably about 85%, and the flow rate should be adjusted seasonally to maintain the temperature in the core of the pile within the active growth range of the fungus, which must be determined for each species. In the case of *C. subvermispora*, the range is approximately 27° to 32°C.

After inoculation, the chips are conveniently collected in large piles. Temperature and humidity control are important for optimal fungal propagation and lignin degradation or modification. Typically, Applicants have determined that practical control can be maintained for piles loaded onto the bottom frame referred to above having dimensions about 40-55 feet high, 100 feet wide and any length. Two 400 foot long piles can accommodate a pulp plant utilizing 600 tons of chips daily. To obtain proper humidity, wet bulb/dry bulb tests can be performed on the influent air. Relative humidity should preferably be maintained at about 70%-90%. Humidification of air by conventional means such as fogging prior to pumping or fanning into the frame ducts is generally necessary

The amount of heat generated in the pile generally requires continuous dissipation by forced air flow even during the winter months in the northern climes.

Incubation times are related to the degree of lignin digestion or modification desired, the type of wood chips being handled, and the particular fungus or combination of fungi being utilized in the process. Useful periods of incubation range from a few days to four weeks. The energy savings to be realized in the subsequent mechanical dispersement phase depend to appreciable extent of cell wall modification caused by the fungus. On the other hand, prolonged incubation results in larger standing inventories of chips and larger on site storage capacity.

Other configurations, in addition to the linear piles formed by the deposition of chips by a moving steamer/inoculator, include circular deposition to form a ring, or deposition in cylindrical silos. It was found empirically that without adequate air flow in the pile, heat tended to build up to unacceptable levels for piles inoculated with *C. subvermispora*, especially in the top 7-12 feet of chips. Construction of a silo taller than 40 to 55 feet would require internal vertical ducting to distribute air upwardly into the pile. The advantage is more compact incubation with conservation of ground space. Enclosure surprisingly was not required, as in the laboratory scale bioreactor. Chip piles were not enclosed or covered, and exposure to the elements did not interfere with the process.

The Applicants have discovered that some wood chip configurations do not require forced-air ventilation. In experiments described in detail in Example 8, an insulated silo (a free-air silo) placed in an environmentally controlled room was used to simulate the conditions of a commercial scale wood chip pile. Specifically, the insulation allowed the wood chips produce and retain heat at rates similar to a

commercial scale wood chip pile. The silo was not cooled by forced-air ventilation. However, as the mass contained within the silo heated, convection currents resulted and air was drawn into the silo through openings in the top and bottom of the silo. The temperature of various zones within the silo was monitored during biopulping runs by the use of thermocouples.

Surprisingly, wood chips were effectively biopulped even when incubated in areas with temperatures outside of the optimum temperature range for biopulping as determined by laboratory scale bioreactor experiments. Energy savings from chips from areas where incubation occurred at below 20°C and above 35°C were similar to energy saving from chips biopulped at optimum temperatures. These data indicate that commercial scale biopulping requires no additional forced-air ventilation. Ventilation from convection currents created by the heating of the interior of the biomass is sufficient to allow biopulping to occur.

When a pile or silo is used for commercial scale biopulping, the configuration of the mass of wood chips is preferably such that the interior of the pile will reach and maintain incubation temperatures of about 20°C to 45°C in two weeks, and most preferably about 35°C. When bipulping is performed in a pile, sufficient mass of wood chips must be provided so that the interior of the pile is adequately insulated. Preferably, the mass of the chips is greater than about 1000 kg.

It will be understood by a person skilled in the art that the dimensions, configuration, and mass of the pile may be varied. However, what is important is that the temperature of the pile reach the preferred ranges. By allowing a gradual increase in temperature over 7 to 10 days by heat accumulated and retained through endogenous biological metabolism, the fungi become conditioned to a higher than optimum temperature.

without loss of biopulping efficiency, or loss of paper strength. The molecular or physiological basis of the conditioning process is not yet understood. It may be that the conditioning is accompanied by induction of heat shock proteins or other metabolites that stabilize the organism. It may also be that at a certain level of lignin-degrading enzyme, activity is maintained in the microenvironment of the chip, even though there may be some decline in viability. The critical element appears to be a chip mass sufficient to result in the natural accumulation of heat as shown by temperature increase. Comparison of simulated conditions in a bioreactor does not achieve the same result, so that the criticality of the chip mass is evident from the Examples. The chip mass also appears not to require an absolute dimensional relationship, although the height of the pile or silo is more significant than the diameter. This is probably related to the "draw" of air, which is needed for metabolism, but not so slow as to accumulate toxic levels of heat prematurely. Since the conditions will vary slightly for the type of wood, the inoculating organism, the nutrient adjuvant, two or three experimental runs can be made to determine variance in the conditions effecting optimal results.

In situations where the type or mix of wood will vary from one run to another, the preferred embodiment will utilize the controlled temperature condition using forced air so that optimization does not need to be revalidated day by day. The static or passive aeration step in biopulping is most effective where the type of wood and the conditions for biopulping do not vary over long periods of time. In these situations, an addition cost savings will be realized.

Wood preparation

As disclosed in U.S. Patent No. 5,055,159 and pending U.S. Patent Application Ser. No. 08/289,429, wood chips are prepared by any conventional technology

to a preferable chip size of between 1/8 and 3/4 of an inch. Any hardwood or softwood species which is commonly used for pulp production may be used here, with preferred species being spruce (*Picea sp.*), southern yellow pine, and aspen.

Biopulping fungi

The fungi used in biopulping must have the capacity to reduce or modify the lignin content of the wood chips during the biopulping process. As disclosed in U.S. Patent Nos. 5,055,159 and 3,962,033, many fungal species in the general class of white-rot fungi have this capacity. Preferred are *Ceriporiopsis subvermispora* and *Phlebia subserialis*, but other white-rot fungi show significant biopulping potential in small-scale trials (such as the laboratory-scale method utilized in U.S. Patent No. 5,055,159) and may be suitable. These include, but are not limited to, species which have demonstrated lignin-degrading or modifying ability such as isolates from the genera *Phlebia, Ceriporiopsis, Dichomitus, Poria, Hyphodontia, Mycoacia, Ganoderma, Phellinus Rigidoporus, Phanerochaete* (also called *Sporotrichum*), *Trametes*, and *Polyporus*.

White-rot fungi vary in several characteristics which influence their suitability in the biopulping process. The most important of these characteristics is the fungus' ability to degrade or modify lignin. Generally, the fungi that have a greater ability to degrade or modify lignin in laboratory-scale trials will be more suitable for biopulping. The lignin-degrading or modifying ability of a fungus can be measured directly by any method known in the art which can quantify the amount of lignin present in wood chips before and after biopulping. Lignin-degrading or modifying ability can also be measured indirectly, by measuring the energy required to convert the wood chips into pulp before and after biopulping with the fungus

in question. U.S. Patent No. 5,055,159 discloses a suitable method for determining energy savings from biopulping. That method entails grinding the wood chips in a mechanical single disk refiner with the feed rate adjusted to maintain a constant load on the refiner motor. Electrical power consumption is measured with an integrated watt-hour meter placed on the refiner. Any other method known in the art may also be used.

As disclosed in U.S. Patent Nos. 5,055,159 and 3,962,033, white-rot fungi vary in their ability to degrade or modify lignin. The two preferred species, *Ceriporiopsis subvermispora* and *Phebia subserialis*, are among the white-rot fungi with the greatest ability to degrade or modify lignin selectively, but many other white-rot fungi show similar potential.

Another factor that influences the biopulping suitability of a white-rot fungus is the optimum growth temperature of the fungus. The process of degrading or modifying lignin generates heat because the enzymatic reactions involved are exothermic. In a large volume of biopulping wood chips, this heat can build up to such an extent that ventilating air is necessary to keep the temperature of the chips at a temperature which is optimum for fungal growth. Therefore, a white-rot fungus that has a high optimum temperature for growth is preferable to one with a lower optimum growth temperature because less ventilation is necessary to cool the chips to the higher temperature. As disclosed in pending U.S. Patent Application Serial No. 08/682,813, *P. subserialis* also grows at a higher temperature than *C. subvermispora*. Therefore, *P. subserialis* requires less ventilation to remove heat than *C. subvermispora*.

Another factor that influences a fungus' suitability for biopulping is whether, during the biopulping process, the fungus produces mycelium on the outside (as well as the inside) of the wood chips, as

described in pending U.S. Patent Application Ser. No. 08/682,813. Fungi that produce mycelium on the outside of the wood chips, such as *Ceriporiopsis subvermispora* are less suitable than those fungi which produce mycelium only inside the wood chips, such as *Phlebia subserialis*. The presence of mycelium on the outside of the wood chips is disadvantageous because the mycelium may interfere with the flow of ventilating air through a large volume of wood chips, making increased ventilating energy necessary to overcome the airflow interference caused by the outside mycelium. This factor is not determinative of a fungus' suitability for biopulping, however, since *Ceriporiopsis subvermispora* produces mycelium on the outside of inoculated wood chips, yet that fungus is quite suitable for biopulping.

Another characteristic of a biopulping fungus which affects the ventilating airflow requirement is the extent to which the fungus decomposes the wood chips during the biopulping process such that the chips may be compressed by the weight of the chips above them. This compression may impede the ventilating airflow, requiring a greater ventilating energy to remove sufficient heat to cool the chips adequately. Between the two preferred species, *C. subvermispora*-treated wood chips are much more compressible than *P. subserialis*-treated chips.

To determine the suitability of a white-rot fungus for biopulping, the various characteristics described above should be weighed together. Thus, for example, *C. subvermispora* is less suitable than *P. subserialis* in the characteristics of having a lower optimum growth temperature, growing on the outside of the chips, and causing more chip compressibility, but superior in lignin-degrading or modifying ability. Overall, *C. subvermispora* is similar to *P. subserialis* in its suitability as a biopulping fungus.

The process of the present invention is suitable for inoculation of wood chips with any fungus. The process may thus be used with delignifying fungi such as those described above, as well as with fungi which do not delignify, such as *Ophiostoma piliferum*, which can reduce the pitch content of the wood chips, as disclosed in U.S. Patent No. 5,476,789. Large scale fungal treatment of wood chips is being practiced in the Cartapip® process of the Clariant Corporation, which is disclosed in U.S. Patent No. 5,476,789. The Cartapip® process does not use a white-rot fungus and does not affect the lignin in the wood. Rather, it removes pitch and controls colored microorganism contamination that consumes bleaching chemicals. In that process, a dried suspension of an isolate of the fungus *Ophiostoma piliferum* is simply mixed with water and sprayed onto wood chips prior to storage in a chip pile. The chips are then stored in the chip pile for more than six days. The present process of treating wood chips and inoculating with *O. piliferum* instead of white-rot fungus is adaptable to such other fungal treatment objectives.

Fungal inoculum and nutrient requirements

The white-rot fungus used for biopulping may be maintained and increased for preparing biopulping inoculum by any method known in the art. In the preferred embodiment, cultures are continuously maintained in serial culture and potato dextrose agar slants. Working cultures are prepared from the stock cultures as needed and refrigerated until used. Potato dextrose agar plate cultures are inoculated from a working culture and incubated at 27°C and 65% relative humidity for 10 days.

In preparing the inoculum, preferred is a liquid inoculum prepared as follows. Potato dextrose broth (4.8 g) and yeast extract (1.46 g) are added to 200 ml of distilled water and mixed well. 100 ml of this

medium is poured into two 1-L flasks. Each flask is autoclaved for 20 min at 121°C. After cooling to room temperature, each flask is inoculated with 10 plugs cut with a number 9 cork bore from a 10-day-old potato dextrose agar plate of the fungal culture. The flasks are then incubated at 27°C at 65% relative humidity for 10 days. Prior to use, the flasks containing the fungal biomass are decanted and washed with sterile distilled water to remove excess medium from the fungal biomass. The fungal biomass is then placed in distilled water and blended in a Waring blender twice for 15 s, each time at high speed. Distilled water is then added to the suspension to make the total volume 100 ml.

Addition of a nutrient adjuvant to the inoculum, as disclosed in pending U.S. Patent Application No. 08/289,429, is preferred, since that addition substantially reduces the amount of inoculum required. Nutrient adjuvants which may be added to the inoculum are molasses, yeast extract, or corn steep liquor, at volumes of between 0.5% and 3%, on a dry weight basis. Preferred is 0.5% unsterilized corn steep liquor. Enough of this inoculum suspension to produce an inoculation rate of 5 g per ton or less of chips on a dry-weight basis is used to inoculate the bioreactor or piles.

The Apparatus

A preferred embodiment for an apparatus for treating wood chips for biopulping is provided in the sixth of the following Examples. Other examples may also embody the principles of the present invention, but Example 6 is believed to best illustrate how the principles of the present invention may be applied toward commercial use. The sixth example shows a preferred embodiment of the apparatus used for treating wood chips in a forty ton capacity.

As shown in Fig. 1, an apparatus 10 for treating wood chips 11 is provided. The apparatus 10 comprises a conveyor 12 that is capable of transporting wood chips 11 from one location to another. In operation of the invention, various structural components may be mounted to the conveyor 12 in order to perform various parts of the treatment process while the wood chips 11 are being transported. In this way, the conveyor 12 makes continuous processing of the wood chips 11 possible. By that it is meant that more than one of the various components of the treatment process may be performed simultaneously. This is contrasted with the previous implementation of the process, in which a single batch of wood chips was taken through the stages of the entire process in sequence. In use, it was found in Example 6 that in addition to performing dual functions, this particular apparatus had additional functionality and efficacy over stationary systems.

The conveyor 12 of the illustrated embodiment is comprised of a first conveyor portion 14 and a second conveyor portion 16. In addition to this configuration, the present invention may include a single continuous conveyor or a number of functionally connected conveyors. The first conveyor portion 14 and the second conveyor portion 16 are functionally connected in such a way that the respective flow rates of wood chips 11 are approximately equal, and individual portions of wood chips passing through the conveyor 12 receive substantially equivalent treatment.

A surge bin 18 is provided near the end of the first conveyor portion 14 and the beginning of the second conveyor portion 16. The surge bin 18 is operatively connected to the first conveyor portion 14 and the second conveyor portion 16. In operation, the surge bin 18 contains a reservoir of wood chips 11 expelled of first conveyor portion 14, thereby buffering the second conveyor portion 16 from flow surges from the first conveyor portion 14. The surge

bin 18 may also have additional uses, such as hydrating or cooling the wood chips 11, as described elsewhere herein in further detail. A feeder 19 functions as an input source and is mounted to the first conveyor portion 14 and may contain a reservoir of wood chips 11.

In the specific experiments set forth in the Examples the discontinuous screw conveyor 12 is interrupted by a surge bin 18. The surge bin 18 permits flexibility in process design by allowing the conveyance to be varied between the heating and the inoculating steps. However, in industrial practice, it may be preferable to integrate the two substantially continuous processes into a single conveyance vehicle.

The conveyor 12 of the preferred embodiment shown in the figures is a screw type conveyor, comprising an auger 20 mounted within a housing 22. This particular configuration of the conveyor has several beneficial features. First, the screw conveyor agitates the wood chips while they are being transported. This is particularly beneficial because in this way substantially all of the surfaces of the wood chips are exposed for treatment. Process steps that involve decontaminating and inoculating the surfaces of the wood chips may be effectively performed while the chips are being conveyed. Second, screw conveyors are commercially available in sizes suitable to applications of this type.

The conveyor 12 further comprises a means for agitating the wood chips 11. As previously stated, the agitating means may be the auger 20 itself. Other types of conveyors having other types of agitating means may be possible according to the present invention. For example, a flat conveyor that includes a vibrating member as an agitating means may sufficiently agitate the wood chips to allow effective treatment. There may be a mechanism added to a conveyor to serve as an agitating

pressurized air or steam jets, for agitating the wood chips within a conveyor. Further, other geometric configurations, such as a double auger screw conveyor, may function adequately according to the present invention.

As shown in Fig. 2, the conveyor 12 may be arranged in such a way that it can be moved along the ground surface in order to create a linear pile of wood chips 11. The wood chips will then form a pile 22 of triangular cross section. In the illustrated embodiment, the second conveyor portion 16 rotates around the end into which the wood chips 11 enter, thereby tracing out a wood chip pile in an arc shape. The apparatus 10 includes a strut 24 on which the second conveyor portion 16 is mounted and the surge bin 18 is fixedly attached to a rotating mount 26. Alternatively, the entire apparatus may be moved in various ways to create piles 22 of various shapes.

The apparatus 10 further comprises a decontaminator, shown in the illustrated embodiment as the heater 28 and the cooler 32. The heater 28 and cooler 32 together serve to decontaminate substantially all of the surfaces of the wood chips so that the designated fungus may act upon the wood chips 11.

The apparatus 10 further comprises a heater 28, shown mounted to the first conveyor portion 14. In the illustrated embodiment, the heater 28 injects a heat transfer medium, steam, into the conveyor 12 by means of a plurality of ports 30 along the length of the conveyor. As further described in Example 6, a longitudinal configuration of ports was shown to function, however many configurations may be well suited to characteristics of different conveyor types or biosystems. As a result of the configuration of the heater 28 and the agitation of the conveyor 12, the steam is evenly dispersed over the surfaces of the wood chips.

The apparatus 10 further comprises a cooler 32, shown mounted to the second conveyor portion 16 near the entrance. The cooler 32 injects a cooling medium, cooling air, into the conveyor 12. The air may be conditioned to a specific temperature and humidity, and filtered to remove impurities, and is again evenly dispersed over the wood chips.

The apparatus 10 further comprises an inoculator 34, shown mounted to the second conveyor portion 16 around the middle portion. The inoculator 34 injects inoculum into the conveyor 12 to inoculate the wood chips 11. The inoculator 34 disperses the inoculum evenly throughout the agitated wood chips moving through the conveyor, resulting in substantially uniform surface treatment.

The apparatus also includes ventilator 36 for aerating the wood chip pile 22. The ventilator is positioned within the interior of the wood chip pile. If the wood chips are merely piled on the ground, the uninoculated wood chips are layered over the top of the pile. As shown in Fig. 3, the wood chip pile is generally covered with a layer of uninoculated wood chips over the inoculated wood chips. In Fig. 1, the plenum of the ventilator 36 is shown in the middle of the wood chip pile 22. The ventilator also includes a ventilation system connected to the plenum for forcing a ventilating fluid, humidified air in the examples, into the plenum. The plenum may have a plurality of openings in its surface for exhausting the humidified air into the wood chip pile 22. Further, the ventilator includes a control system that controls the volumetric flow of ventilating fluid into the wood chip pile 22. As discussed in the examples, a typical incubator was used in the laboratory scale experiments. However, according to the invention, a commercial sized regulated wood chip incubator is effectively created from such a configuration of the wood chip pile 22.

In the experiments described in Example 8, a free-air silo was used for biolpulping. The free-air silo differs from other biopulping devices in that there is no forced-air ventililation. Ventilation is provided by convection currents created by heating in the center of the biomass incubating in the free-air silo.

A schematic drawing of the free-air silo is presented in Figure 14. The free-air silo 100 comprises a cylindrical vessel 104 for containing a mass of material. Preferably, the cylindrical vessel is insulated with an insulating material 108 having an R value of about 22. The cylindrical vessel 104 includes an upper opening 108 and a lower opening 112. The size of the upper and lower openings 108 and 112 may vary, but must be sufficient to allow air to be drawn into the silo by convection currents, thereby maintaining the mass in the silo at a temperature range of about 20°C to 45°C. Preferably the top opening has thereon a baffle 114 for preventing environmental air currents from drawing air through the vessel 104. The cylindrical vessel includes a means 116 for supporting the mass in the incubator above the lower opening 112. Preferably, the supporting means is a perforated grid, such as hardware cloth. The cylindrical vessel also includes a plurality of thermocouples 120 for monitoring the temperature of the mass. The cylindrical vessel 104 also includes a drain 124 for removing liquid that accumulates in the bottom of the cylindrical vessel 104.

Examples

Example 1. Scaling up wood chip decontamination and inoculation methods.

Before wood chips are inoculated with a white-rot fungus for biopulping, they must be treated to eliminate contaminating indigenous microorganisms present on the chips. As disclosed in U.S. Pat. No. 5,460,697, this decontamination step is performed

because the indigenous microorganisms grow rapidly, inhibiting delignification by the white-rot fungus, and consuming cellulose, which results in the loss of paper strength. Decontamination can be accomplished using chemicals such as sulfites, disclosed in U.S. Pat. No. 5,460,697. However, concern over the large scale use of chemical agents and their potential impact on the environment may limit this application.

Decontamination can also be accomplished by using the heat provided by steam. As disclosed in U.S. Pat. App. Serial No. 08/682,813, this can be accomplished by exposing wood chips to steam at atmospheric pressure for 10 minutes. This treatment, conducted in the bioreactors disclosed in U.S. Pat. No. 5,055,159, was such that the force of the steam injection did not displace the chips.

A series of decontamination or biosuppression experiments were performed in 1-L bioreactors with 50-100 g of loblolly pine (*Pinus taeda* L.) wood chips. The chips were obtained from Union Camp Corporation of Savanna, GA. The wood chips were placed in plastic bags in 55 gallon drums and frozen until used to prevent growth of contaminating microorganisms. The chips to be treated were thawed and thoroughly mixed to obtain uniform samples. In these experiments, steam was injected forcefully into the bioreactors such that the chips agitated vigorously. After the chips cooled, the bioreactors were inoculated with the white-rot fungus *Ceriporiopsis subvermispora* strain L-14807 SS-3 by the following method.

The fungus was obtained from the Center for Forest Mycology Research at the USDA Forest Service, Forest Products Laboratory, Madison, Wisconsin. Cultures were continuously maintained in serial culture and potato dextrose agar slants. Working cultures were prepared from the stock cultures as needed and refrigerated until used. Potato dextrose agar plate cultures were

inoculated from a working culture and incubated at 27°C and 65% relative humidity for 10 days.

In preparing the liquid inoculum, potato dextrose broth (4.8 g) and yeast extract (1.46 g) were added to 200 ml of distilled water and mixed well; 100 ml of this medium was poured into two 1-L flasks. Each flask was autoclaved for 20 min at 121°C. After cooling to room temperature, each flask was inoculated with 10 plugs cut with a number 9 cork bore from a 10-day-old potato dextrose agar plate of the fungal culture. The flasks were then incubated at 27°C at 65% relative humidity for 10 days. Prior to use, the flasks containing the fungal biomass were decanted and washed with sterile distilled water to remove excess medium from the fungal biomass. The fungal biomass was then placed in distilled water and blended in a Waring blender (VWR Scientific) twice for 15 seconds, each time at high speed. Distilled water was then added to the suspension to make the total volume 100 ml. Enough of this inoculum suspension to produce an inoculation rate of 5 g per ton of chips on a dry-weight basis was used to inoculate the bioreactor. The wood chips in the bioreactors were inoculated with this inoculum amended with 0.5% unsterilized corn steep liquor (dry weight basis) and an appropriate amount of sterilized water. Corn steep liquor was obtained from Corn Products, Summit-Argo, Illinois. After inoculation, the bioreactors were incubated at room temperature and observed periodically for visible contamination.

Under these conditions, very short steaming times were effective in decontaminating the wood chips sufficiently. All reactors steamed for 15 seconds to 5 minutes were successfully decontaminated. The tumbling action of the chips exposes the entire surface of the chips to the steam, since 21-L aerated static bed bioreactors (described in Kirk et al., *U.S.D.A. Forest Service, Forest Products Laboratory Research Paper* FPL-RP-523) required at least 2.5 minutes of steaming to

effectively decontaminate the wood chips when the chips were not positionally disturbed during steaming.

In these experiments, Applicants found that only the chip surface requires exposure to temperatures to obtain suppression of the contaminating microorganisms. Raising the temperature of the entire chip, as during long exposures to atmospheric steam or autoclaving, increases the cooling load required to cool the chips sufficiently to inoculate them with the biopulping fungus, without providing any process advantage.

The biopulping fungus, *C. subvermispora*, has an optimum growth temperature of 27°C to 32°C. However, the chips were cooled to a temperature of 40-50°C without loss of viability. Further cooling after the application of the fungus to the chips brings the temperature to the optimum growth temperature.

These experiments show suppression of contaminating organisms is achieved by a 15 sec treatment with steam at atmospheric pressure if the chips are tumbled sufficiently to expose the entire chip surface to the steam.

Example 2. Ventilation in *Ceriporiopsis subvermispora*-inoculated wood chip piles.

Experiments were designed to determine whether the biopulping chip pile requires ventilation to remove excess heat created when lignin is metabolized by the biopulping fungus.

An experiment utilizing a 600 kg (wet) wood chip pile showed that ventilation is required to control temperature when biopulping with *Ceriporiopsis subvermispora* in large wood chip piles. The chips were obtained and stored as described in Example 1. After thawing, the chips were autoclaved. After cooling overnight, the chips were inoculated with inoculum prepared as described in Example 1. Mixing of the inoculum was done in a large rotating "V" mixer with an efficient mixing capacity of 5 drums of chips (about

275 gal). The inoculated chips were transferred to a pile using a drum as a transfer vessel. The pile was covered with about 60 kg of autoclaved, uninoculated chips and was divided into the zones shown in Fig. 3 for analysis. To monitor the temperature in these zones, thermocouples were placed in the positions of the pile shown in Fig. 4.

Fig. 5 shows the temperature profiles of the four zones of the pile during the 2 week incubation. The Top and Center zones of the pile reached nearly 42°C by the sixth day of the trial as a result of heat generated by the fungal metabolism. This temperature is well above the range for optimum growth of *C. subvermispora* (27-32°C). Contaminating fungi were visible throughout this area after the incubation ended. In contrast, the outer shell of the pile (Fig. 6) maintained an optimum temperature range for fungal growth during most of the incubation period. There was no visible contamination in the outer shell.

At the end of the 2 week incubation period, chips from the outer shell of the pile, and uninoculated control chips were refined in a 300 mm diameter mechanical atmospheric disk refiner to measure electrical energy consumption during refining. Chips from the outer shell of the pile used about 36% less energy than the control chips.

These results illustrate several points regarding the growth of *C. subvermispora* in larger piles. First, the fungus is not self-regulating and can quickly generate sufficient heat to raise the temperature above the optimum temperature for fungal growth (27-32°C). Also shown is that the natural draft generated by the heat production is not sufficient to cool the pile to keep the fungus within its optimum range for generalized biopulping wherein all conditions are to be uniform. In larger piles, the heat buildup would probably be even greater. These results demonstrate

the need for active cooling of the pile, except where the pile or silo can be optimized for natural conditioning of fungi for effective biopulping at elevated temperatures.

Example 3. Use of tubular reactors to determine ventilation requirements of *Ceriporiopsis subvermispora*-inoculated wood chip piles.

Forced air is an effective way to remove hot air from within a chip pile. Air flow through a chip pile which is generating heat from fungal metabolism, however, is complex. The use of tubular bioreactors is useful in simplifying these air flow patterns to obtain engineering and kinetic data necessary for scaling up the process. One of these tubular reactors, a PVC reactor which is 20.3-cm diameter and 1.0-m high, was disclosed in pending U.S. Patent Application Serial No. 08/682,813. This reactor holds 6.0 kg of dry wood chips and is illustrated in Fig. 6. The bottom of this reactor has a polyethylene grid containing 6-mm holes through which air could be introduced into the reactor, through the wood chips inside, and out the top, which had an air hole in it. Temperature measurements could be made during biopulping runs using thermocouples inserted along the length of the reactor as indicated in Fig. 6.

A series of experiments were performed using the PVC reactor described above. These experiments utilized two strains of *C. subvermispora*, SS-3 and CZ-3, and various rates of air flow supplied to the bottom of the reactor during the biopulping incubations. The chips for each experiment were decontaminated and inoculated in four of the laboratory scale bioreactors disclosed in U.S. Pat. No. 5,055,159 (each containing 1.5 kg chips) using steam at atmospheric pressure for 10 minutes, then cooled to room temperature prior to inoculation. Inoculum was prepared, and inoculation was performed as described in Example 1, except that in

Experiments 1 and 2, the corn steep liquor was sterilized by autoclaving. After inoculating four laboratory scale bioreactors, the bioreactors were vigorously shaken for uniform mixing, and the inoculated chips were poured from the bioreactors into the PVC reactor, which was then capped and sealed. The reactor was insulated and placed into an incubator at 27°C for 2 weeks. Nearly saturated humidified air at 27°C was supplied to the bottom of the reactor at a rate ranging from 0.055 to 0.220 volume of ventilating fluid (humidified air)/volume of wood chips/minute (v/v/m), which is equivalent to 4 to 16 ft^3/hr in the PVC reactors. At harvest, energy savings resulting from the biopulping were determined as in Example 2. Table 1 summarizes this data. The energy savings was generally highest at an air flow of 0.110 v/v/m. At the lower air flow of 0.055 v/v/m (Experiments 1 and 2), higher energy savings was achieved by chips at the bottom section of the reactor than those at the top, but at higher air flows (Experiments 6-11), the differences in energy savings among the areas of the reactors was much less. The temperature profiles during these runs are shown in Figs. 7-9. Fig. 7 shows the temperature profiles for Experiment 2. After about 4 days, when fungal growth and metabolism were greatest, the temperature increased rapidly in the upper sections, to 38°C in the top section. This indicates that the air flow of 0.055 v/v/m was not sufficient to remove enough heat from the upper sections to keep the temperature at the optimum for *C. subvermispora*. When the airflow was doubled to 0.110 v/v/m, as in Experiment 6, shown in Fig. 8, the temperature in the upper sections of the reactor was maintained at a more optimal level. Finally, at the highest airflow, 0.220 v/v/m, utilized in Experiment 9, shown in Fig. 9, the temperature was moderated further. However, at the highest airflow (0.220 v/v/m), the energy savings was lower from chips at the bottom

section, perhaps because the bottom chips dried out. This deleterious effect can be overcome by fogging the inlet air. Reversing the direction of air flow periodically may also increase energy savings when the inlet air temperature is 16-20°C, as revealed by a comparison of Experiments 7 and 8.

The next object is to progressively scale up the process to obtain an understanding of the problem of temperature control and heat management as a function of biomass.

Approximately 160 kg of loblolly pine (*Pinus taeda* L.) chips (dry basis) were prepared, inoculated and mixed as in Example 2. The inoculated chips were then transferred to the silo reactor using a 55-gallon drum as the transfer vessel. The flow rate and the temperature of the introduced ventilation, using nearly saturated air, was adjusted to maintain the proper temperature range throughout the reactor. Fig. 10 shows the temperature profiles in a silo reactor run. During the first day, the temperature at all locations in the silo increased to 30°C, the inlet air temperature. After 2 days, a rapid increase in the heat production rate required an increase in the air flow rate from the initial 0.119 v/v/m to 0.441 v/v/m, and a decrease in the inlet temperature to 28°C, as indicated in Fig. 10. Two additional changes in the inlet temperature, as indicated in Fig. 10, kept the temperature throughout the reactor to the optimum range of growth for *C. subvermispora*. After harvest, energy savings were determined by the method described in Example 2. The resulting pulp was made into paper and that paper was tested for strength property improvements over uninoculated control chips. Table 2 shows the results of those tests. The energy savings exhibited from chips throughout the silo was similar to energy savings achieved in the smaller PVC reactor as well as in 2-week incubations in laboratory bioreactors

disclosed in U.S. Patent No. 5,055,159, and U.S. Patent Application Serial Nos. 08/289,429 and 08/682,813.

The silo experiments show that adequate environmental conditions can be maintained throughout a height of 2 m during a biopulping run with *C. subvermispora* by adjusting the ventilation air flow and temperature. This was surprising in light of pending U.S. Patent Application Serial No. 08/682,813, which indicated that the prolific production of aerial hyphae by *C. subvermispora* significantly interferes with air flow, as measured by the pressure drop through a PVC reactor column. As discussed in the patent, *Phlebia subserialis* is considered superior to *C. subvermispora* because *P. subserialis* does not form aerial hyphae. Air flow through chips inoculated with *P. subserialis* is thus less impeded (as measured by the pressure drop over the length of the column) than through chips inoculated with *C. subvermispora*.

In a commercial size chip pile or silo (40 tons or more), the weight of the chips may compress the pile such that the flow of air through the pile may be impeded. Such interference of the air flow might inhibit the ability of the air to remove the heat generated in the pile. Greater compression is expected in the fungally treated chips because of the softening action of the fungus. A certain amount of settling is observed in the bioreactors during the 2-week incubation (approximately 5%). This is expected to be of greater concern in the larger chip piles. To study this, a cylindrical bioreactor was fabricated which was capable of being placed in a press and loaded to simulate the weight that would be placed on chips at the bottom of a large pile or silo. A piston with a perforated plate was used to compress the chips while allowing free flow of the air through the column. In this way, ventilation flow studies could be done while the chips were under a load. Chips, either untreated controls or inoculated as described in Example 3, were

placed in the reactor to a height of slightly less than 1 m. After incubation of the inoculated chips, the piston was placed on top of the chips, and the reactor was placed in a press that used a constant rate of displacement. The chips were compressed at a rate of 0.25 cm/min, and the load and compression readings taken at 222-N intervals. During compression, air was blown upwards through the column of chips at a space velocity of 0.88 cm/sec. A maximum load of 4450 N was used.

Fig. 11 shows the compression of the fungally treated wood chips and untreated chips as a function of the load that is applied. A load of 100 kPa is equivalent to the weight of wet wood chips (50% moisture) approximately 30 m high. The chips treated with *C. subvermispora* are highly compressible, reaching compressions of more than 15% under a load of 80 kPa. At the same load, control chips only compressed about 7%. *P. subserialis*, on the other hand, did not soften the chips to such a large degree as *C. subvermispora*. The chips treated with this fungus only compressed approximately 1% to 2% more than the untreated control chips. Because *P. subserialis*-treated chips compressed less than *C. subvermispora*-treated chips, the air flow through a *P. subserialis* biopulping run should be less restricted, thus reducing the cost of ventilation.

Example 4. Ventilation requirements in various sized *Ceriporiopsis subvermispora*-inoculated wood chip piles.

Both small and large chip piles were used to investigate the efficacy of biopulping under less controlled conditions. Small chip piles (Fig. 12) typically consisted of approximately 30 kg (dry) of inoculated chips (prepared as in Example 1) covered with 10 kg of uninoculated chips, resulting in a pile approximately 0.65 m high. Applicants placed inoculated chips onto an insulated pad and monitored the internal temperature of the pile near the top of

the pile. The chips were left in the piles for 2 to 3 weeks, and at harvest, refined and the energy savings determined as described in Example 2. The only source of ventilation for these piles was the natural draft created by the heat generation within the pile and air infiltration at the surface. With piles of this size, overheating of the pile was not a problem, as this natural air exchange was sufficient to keep the pile within the levels necessary for fungal growth. Temperatures ranged from 27° to 36°C during the incubations. Chips in these piles were heavily colonized throughout, achieving energy savings of greater than 30%, which is comparable with that obtained under controlled conditions using PVC or silo bioreactors.

Large piles, containing between 300 and 600 kg (wet) chips were constructed and inoculated as described in Example 2. Ventilation was provided as indicated in Fig. 4 with sterile humidified air.

Fig. 13 shows the temperature of different regions of a 600 kg pile during a biopulping incubation where the ventilation rate was adjusted to maintain the upper temperature of the pile at approximately 32°C or below. This run can be directly compared to Example 2, describing a 600 kg pile which was not ventilated. Here, the ventilation was initially maintained at a very low level, sufficient to maintain positive pressure in the pile. As Fig. 13 shows, the initial temperature increase as a result of the growth of the fungus occurred between the third and fourth day of the trial, and at this time the air flow rate was increased. Although the top and the center were initially the hottest regions of the pile, after the ventilation rate was increased, the center was cooled to the optimum temperature for fungal growth. Only minimal drying of the chips was noted at the center of the pile near the air inlet. The amount of air for effective cooling of the pile was about 0.022 to 0.136

v/v/m (40-244 ft^3/h). With this approach relatively uniform biopulping action throughout the pile could be achieved. Energy savings of about 30% was obtained.

Moving from enclosed bioreactors to chip piles increases the possibility of contamination from several sources, including the humidified air, the inoculum, the unsterilized corn steep liquor, and the outside air. Chip pile handling and the natural draft entering from the base or edges of the piles might also introduce contaminants into the pile. However, if the fungus starts colonizing chips early, as occurs during optimum growing conditions, no contaminants are evident and biopulping efficiency is not affected.

Example 5. The biopulping of a four ton wood chip pile.

This trial was performed indoors with four tons of Spruce (*Picea sp.*) wood chips. Decontamination and inoculation of the chips was performed using an apparatus consisting of 2 screw conveyors with a surge bin between them. Treatment proceeded at a rate of 10 kg (oven dry) of chips per minute. The chips were shoveled into the hopper of the first screw conveyor where the chips were steamed at atmospheric pressure in order to decontaminate them. This first screw conveyor was 16 ft long and 7 inches in diameter. The chips were in this conveyor for approximately 35 sec. Steam was injected into the shell of this first conveyor through eight injection ports along the conveyor at a total rate of about 1.5 to 2.0 kg of steam per minute. Six of the eight injection ports were located on the top of the conveyor spaced at two foot intervals. The other two ports were on the sides of the conveyor, 180° apart.

From the top of the screw conveyor, the chips dropped into a surge bin that was 3 ft in diameter and 5 ft high, where the chips were retained for approximately 3 to 10 min. The bin, which was well

insulated, was vibrated to aid in the downward movement of the chips and had steam injected into the bottom at a rate of approximately 3.0 to 4.0 kg of steam per minute.

From the bottom of the surge bin, a second screw conveyor picked up the chips and transported them to the top of the incubation chamber. This second conveyor was 25 ft long and 10 inches in diameter. The chips were in this second conveyor for approximately 1 min. In the lower part of this second conveyor, filtered air was blown across the chips in order to cool the chips by evaporative cooling. The air was supplied by a 3 hp blower with a capacity of approximately 2000 ft^3/min. This blower blew air into the center of a filter box which contained three banks of three filters each. The first two banks consisted of pleated furnace filters and the third bank contained higher efficiency foam home furnace filters. The air flow rate was approximately 1500 to 2000 ft^3/min. After cooling, the inoculum, prepared as in Example 1, was pumped into the shell of the conveyor and onto the chips at the midpoint of the conveyor. The final 10 ft of the conveyor thoroughly mixed the chips with the inoculum.

From the top of the second conveyor, the chips dropped into the incubation chamber, which was an 8 ft high insulated room approximately 11 ft by 14 ft. Dropping from the second conveyor, the chips impacted a spinning "chip flinger," which distributed the chips relatively evenly over the entire chamber. The chips were held approximately 4 inches above the floor of the chamber by a platform with the surface of 1/2 inch hardware cloth. Three air ducts fed into this air space to supply the cooling air to the chips. One duct fed to the center 4 ft by 4 ft area, while the other two ducts fed the surrounding area. The two areas were separated by a 4 inch high baffle that reached to the bottom of the hardware cloth platform.

Filtered air was supplied by the same pump and filter apparatus which supplied the filtered air used to cool the chips before inoculation. After the air left the filter box, steam was injected into the air in order to humidify and warm the air. After humidification, the air was cooled back to the desired wet bulb temperature, causing condensation and saturating the air. This cooling was done indirectly using tap water flowing through finned heat-exchanger tubes. The air was then heated indirectly with steam through finned heat-exchanger tubes to the desired dry bulb temperature. Wet and dry bulb temperatures of 27° and 29°C, respectively, were commonly used. The air flow rates during the incubation were between 0.173 v/v/m and 0.812 v/v/m. Thermocouples placed in several areas of the pile monitored the temperature.

After 2 weeks of incubation, a sample mixed from several areas of the pile was processed as in Example 2 and strength improvements over uninoculated control chips in paper made from the sample were determined. The sample from this incubation required 26% less energy to produce pulp than a control sample, which is comparable to data using smaller incubators, under more controlled conditions. Similarly, improvements in the burst index, tear index, and tensile index of 21, 34, and 14% over the control were also similar to data using other bioreactors. Thus, these scale up methods were successful in reproducing the benefits achieved using smaller bioreactors.

Example 6. The biopulping of a forty ton wood chip pile.

The process was scaled up ten-fold to forty tons of spruce chips in an incubation which was conducted outdoors in Madison, WI during October of 1996. Forty tons of chips were treated at a rate of 30 kg (oven dry) chips per min. An equivalent design of two screw convevors separated by a surge bin, described in

Example 5, was utilized, except the apparatus' dimensions were greater. The first screw conveyor was 25 ft long and 10 inches in diameter. The chips were in this first conveyor for approximately 65 seconds. Steam was injected at about 11.3 kg of steam per minute through 18 injection ports evenly spaced along the top of the conveyor. The surge bin was 4.5 ft in diameter and 10 ft high. From the top of the first conveyor, the chips dropped into the surge bin where they stayed for approximately 2 to 10 minutes. The bin, which was well insulated, was vibrated to aid in the downward movement of the chips. From the bottom of the surge bin, the second screw conveyor, also 25 ft long and 10 inches in diameter, picked up the chips and transported them up the conveyor. The chips were in this second conveyor for about 65 seconds. In the lower part of this second conveyor, filtered air was blown across the chips in the conveyor at a rate of about 1500 to 2000 ft^3/min. After cooling, the inoculum was pumped into the shell of the conveyor and onto the chips at the midpoint of the conveyor. The final 10 feet of the conveyor thoroughly mixed the chips with the inoculum. All other aspects of this decontamination and inoculation procedure were the same as in Example 5.

From the top of the second conveyor, the chips dropped onto the pile from a height of approximately 15 feet. The entire treatment apparatus was moved to produce a linear pile with a triangular cross section. The pile was approximately 13 ft high, 28 ft wide, and 67 ft long. A second layer of chips about 1 ft thick was placed on top of the treated chips. These chips, while treated with the fungus, did not contain the corn steep liquor as a nutrient. As with the 4-ton trial described in Example 5, thermocouples were placed in several areas throughout the pile to monitor temperature.

An air distribution system was in place under the pile that could supply up to 8000 ft^3/min. The blower

blew air into the center of the filter box described in Example 5. After leaving the filter box, the air entered a mist chamber into which hot water was sprayed in order to warm and humidify the air. Steam was mixed with water in order to produce the hot water, and the temperature of the air was controlled by controlling the flow of steam. This temperature was near the desired wet bulb temperature of the air. The air was then reheated indirectly with steam through a heat-exchanger tube to the desired dry bulb temperature. Wet and dry bulb temperatures of 27° and 29°C, respectively, were commonly used. The aeration rate was adjusted to maintain the pile temperature to the optimum range for the fungus. It varied between 0.389 and 0.705 v/v/m.

Energy savings and improvements in paper strength properties were determined as in Example 5. The biopulped chips from this 40-ton incubation required 40% less energy to produce pulp than a control sample, which is comparable to data using smaller incubators, under more controlled conditions. Similarly, improvements in the burst index, tear index, and tensile index of 22, 35, and 9% over the control were also similar to data using other bioreactors.

Summaries and Conclusions - Examples 1-6

Tables 3 - 5 compares important factors that varied as the biopulping process was scaled up. In Table 3, the air flow rates required to cool the 4-ton pile was similar to that required for the 40-ton trial, indicating that further scaleup would not require a significant increase in this rate.

Although pending U.S. Patent Application Serial No. 08/682,813 indicated that *Ceriporiopsis* was inferior to other fungi for biopulping because that fungus produces aerial hyphae on the wood chips which interferes with ventilation air flow, *Ceriporiopsis* nonetheless proved to be quite suitable in the large

scale trials. Other fungi such as *Phlebia* or other white rot fungi should therefore be suitable in this process, and may even require less ventilation.

Table 1 - Experiments with PVC reactors. All experiments ran for 2 weeks, using *Ceriporiopsis subvermispora*.

Exper. No.	Strain	Notes	Energy savings (%) top	middle	bottom
1	SS-3		18	6 (avg. 16.5)	24
2	CZ-3		3	12 (avg. 16)	29
6	CZ-3	Inlet temperature 27°C	21	31 (mixed sample: 26)	35
7	CZ-3	Inlet temperature 16-20°C		mixed sample: 21	
8	CZ-3	Inlet temperature 16-20°C, flow reversed occasionally		mixed sample: 25	
9	CZ-3	Inlet temperature 27°C	25	23	18
10	SS-3	Inlet temperature 27°C		mixed sample: 29	
11	SS-3	Inlet temperature 27°C, flow reversed occasionally		mixed sample: 28	

Table 2. Energy savings and improvements in strength properties from 2-week silo run. *Ceriporiopsis subvermispora* SS-3 used on loblolly pine chips. Energy savings and strength property improvements are based on untreated control values.

Silo sections	Energy savings (%)	Improvements (%) Burst index	Tear index	Tensile index
Top	23	0	75	22
Middle	31	0	81	25
Bottom	31	0	76	19

Table 3. Aeration Rates at different scales.*

Reactor	Reactor Volume (ft^3)	Minimum Aeration (v/v/m)	Maximum Aeration (v/v/m)
Laboratory bioreactor	0.7	0.022	0.088
PVC reactor	1.1	0.059	0.235
Silo reactor	29.5	0.119	0.441
4-ton trial	924	0.173	0.812
40-ton trial	9100	0.389	0.705

*aeration rate may be varied from .02 to .8 vol/vol/min

Table 4. Steaming rates at different scales.

Reactor	Chip Flow Rate (kg/min)	Steam Flow Rate (kg/min)	Steam Enthalpy (kJ/kg)	Specific Steam Rate (kg/kg chips)	Specific Steam Heat (kJ/kg chips)
Laboratory bioreactor	0.15	0.1	1776	0.68	1210
Small Conveyor	10	2.0	2660	0.20	530
Small Conveyor and Surge Bin	10	5.4	2600	0.54	1400
Large Conveyor	32	11.3	2400	0.35	850

Table 5. Inoculation parameters for the 4- and 40-ton trials.

	4-ton trial	40-ton trial
Chip Flow Rate	10 kg/min	30 kg/min
Inoculum		
Fungus	5 g/ton	5 g/ton
Fungus suspension	0.153 kg/ton	0.153 kg/ton
Corn Steep Liquor	10 kg/ton	10 kg/ton
Water	130 kg/ton	130 kg/ton
Inoculum Flow Rates	1.40 kg/min	4.24 kg/min
	0.36 US gal/min	1.10 US gal/min
Total Used		
Fungus	20 g	200 g
Fungus suspension	0.61 kg	6.1 kg
Corn Steep Liquor	40 kg	400 kg
Water	520 kg	5200 kg
Volume Used		
Fungus suspension	0.16 gal	1.62 gal
Corn Steep Liquor	8.4 gal	83.9 gal
Water	137 gal	1370 gal

Example 7. Summary of Bioreactor Studies.

Data (based on bioreactor studies) presented in Table 6 clearly demonstrate that the optimum growth temperature for *Ceriporiopsis subvermispora* is between 27-32°C. At both temperatures, 30% energy savings were realized and significant improvements in strength properties (Burst and tear index) were noted compared to the control. Results were also found to be comparable at 27 and 32°C. All of our previous inventions refer to these optimum growth temperatures for *C. subvermispora*. This fungus does not perform biopulping in bioreactors when incubated at 35-39°C; and does not perform biopulping effectively at temperatures lower than 27°C.

Table 6. Effect of temperature on biopulping efficacy of *Ceriporiopsis subvermispora* on loblolly pine chips

Temperature	Energy Savings	Burst Index	Tear Index
Control	-	0.57	1.47
Treatment (17°C)	7	0.53	1.87
Treatment (22°C)	18	0.61	2.24
Treatment (27°C)	30	0.66	2.52
Treatment (32°C)	30	0.61	2.52
Treatment (35°C)	0	-	-
Treatment (39°C)	0	-	-

Bioreactors steamed for 10 min., cooled to room temperature, inoculated with 5 g/ton of fungus in presence of 0.5% corn steep liquor, and incubated for 2 weeks at different temperatures.

Example 8. Biopulping in Silo Reactors in the Absence of Forced Air-flow.

A silo type bioreactor (Free-Air Silo, see Fig. 14) was used in the following example. The free-air silo was built to measure the growth characteristics of

fungi and to assess the effect of heat build-up on chip piles naturally aerated or ventilated without forced air.

The free-air silo comprised a cylinder with an inside diameter of 30 inches inside and which was 82 inches tall. The bottom of the cylinder supports a perforated grid + 1/2 inches above the floor of the vessel. Air enters the cylinder from the bottom via a 6 inch 90 degree plumbing elbow. Air exits the top through a 6 inch hole in a wooden cover which rests on a rubber seal. The restriction on top is to make the exposure on the top of the silo similar to that on the bottom. This prevents air blowing across the top to pull air through the silo like a chimney. The outside of the vessel has an insulation blanket of R22 value.

Major differences from powered (previous) silos include: A larger opening on bottom (6 inch v. 2 inch) so friction doesn't inhibit the flow of air through silo; a top baffle on free air silo to minimize any extraneous air effects on the silo; and, slightly better insulation is employed because all heating (and cooling) is accomplished by the fungus and natural convection.

Biopulping runs in the free-air silo were conducted in a conditioning room. The purpose of this room was to provide a stable environment in terms of temperature for scale up experiments. This was necessary for the study of the effects of heat generated by the microorganisms, which could be partially masked or complicated by the wide temperature variations in the pilot plant.

The conditioning room is a chamber 6 feet long by 9 feet 6 inches tall. It has sufficient room to house one silo reactor plus a recording device and leave enough space to ensure proper air circulation.

Air temperature in the room is properly controlled. A small squirrel cage blower circulates air through a heat exchanger (Radiator) which has hot

and cold water pumped through it which is automatically temperature controlled by an electronic thermocouple regulator. Air passes through this radiator and into the room via ducts that extend from the floor to the ceiling. The large ducts are meant to minimize any floor to ceiling stratification and also to keep air velocities low, both of which can adversely affect how air moves through the silo.

Very light framing on sides and along floor (1x4) inches covered by R5 insulation value foam board on sides and top. The sides are also sheathed in wafer board to protect the insulating value of the enclosure. The front of the chamber contains the door which is 4 foot wide and split into two panels. The lower panel is 4 feet wide by 6 feet 8 inches high, directly above this is the second door panel which is 4 feet wide by 3 feet 6 inches tall. When recording data or examining the experiment, only the lower door is usually used. The doors and opening are gasketed to prevent uncontrolled outside air from entering.

In the first free-air silo biopulping run, 160 kg spruce chips (dry weight basis) were steamed for about 40 seconds and dropped into a "V" mixer. After cooling, a suspension containing fungus (5 g per ton on a dry weight basis), unsterilized corn steep liquor (0.5% on a dry weight basis), and water was added to the mixer. The inoculated chips were rotated in V mixer for uniform mixing. These inoculated chips were dropped into a non-aerated silo. Six thermocouples were placed (Fig. 14) to monitor the temperature profile during two-week period (Fig. 15).

After two-week of fermentation, three samples of fungus-treated chips were collected; one sample from the location of thermocouple 2, a second sample from the location of thermocouple 4, and a third sample from the location of thermocouple 6 (see Fig. 14). These samples were refined to measure the electrical energy consumption. Data presented in Table 7 show that

sample 1 (thermocouple 2) saved 29% energy, sample 2 (thermocouple 4) saved 38% energy, and sample 3 (thermocouple 6) saved only 13% energy. The average energy savings from this entire silo was about 27% which is very close energy savings from laboratory scale biopulping experiments.

Table 7. Biopulping of *Ceriporiopsis subvermispora* on spruce chips in a silo reactor

Sample Identification	Energy savings over the untreated control (%)
Sample 1 (thermocouple 2)	29
Sample 2 (thermocouple 4)	38
Sample 3 (thermocouple 6)	13

These results from silo studies were surprising because of the following unexpected relationship between energy savings and temperature profile in the silo:

1. Fungus-treated chips (sample 1) collected from the location of thermocouple 2 saved 29% energy. However, this thermocouple 4-5 days out of 14 days after inoculation was exposed to more than 35°C (Figure 16). According to bioreactor studies, the fungus should not perform biopulping at or above 35°C (Table 6).

2. Fungus-treated chips (sample 6) collected from the location of thermocouple 6 saved only 13% energy. However, this thermocouple was exposed to 27-30°C for the entire two-week period (Figure 6). According to bioreactor studies, the fungus should have performed biopulping effectively and would have saved 29-30% energy (Table 6). Pulps from fungus-treated chips (sample 1) from the location of thermocouple 2 were also made into paper and tested for strength properties. Significant

improvements in strength properties were noted (Table 8) besides energy savings (Table 7).

Table 8. Strength properties improvements due to *Ceriporiopsis subvermispora* in silo

Treatments	Burst Index	Tear Index
Control	1.15	4.22
Treatment	1.51	4.62

In subsequent studies, we tried to simulate the temperature profile of thermocouple 2 in bioreactors. Temperature profiles of thermocouple 2 and bioreactors are shown in the following Table 9.

Table 9. Temperature profiles of thermocouple 2 in a silo, and simulation of these temperatures in bioreactors.

Days after inoculation	Temperature (°C)		
	Thermocouple 2 (silo)	Bioreactor (A)	Bioreactor (B)
1	28.8	27	27
2	29.5	27	27
3	27.8	27	27
4	28.8	27	27
5	31.1	27	27
6	32.8	32.8	27
7	35.1	35.1	27
8	38.4	38.4	27
9	38.4	38.4	27
10	38.7	38.7	27
11	37.8	38.5	27
12	38.5	39.0	27
13	39.0	40.2	27
14	40.2	40.2	27

Bioreactor B was exposed to 27°C for the entire two-week period and used as a control. Bioreactor A was initially exposed to 27°C for five day, and then exposed to similar higher temperature as those of thermocouple 2. Following results were obtained from these studies (Table 10).

Table 10. Energy savings and strength improvements during temperature simulation

Parameters	Thermocouple 2	Bioreactor (A)	Bioreactor (B)
Energy savings (%)	29	10	33
Burst index (control)	1.15	1.12	1.12
Burst index (treatment)	1.51	1.18	1.44
Tear index (control)	4.22	4.17	4.17
Tear index (treatment)	4.62	4.01	4.43

When bioreactor B was incubated at 27°C for two weeks, 33% energy savings were realized along with improvements in paper strength properties. However, when bioreactor A was exposed to temperatures similar to those of thermocouple 2, only 10% energy savings were observed and no improvements in paper strength properties were noted. In silo studies where fungus-treated chips were collected from the location of thermocouple 2, 29% energy savings were noted and improvements in strength properties were realized. These results were surprising. These results clearly indicate that fungus performs differently, but favorable on a larger scale. The reason for these results is not known at the moment. However, one possible explanation could be that the fungus tolerates higher temperature on a larger scale may be because the fungus produces some metabolites that make the fungus more temperature resistant.

Another silo experiment was conducted which demonstrated that biopulping may be effective at temperatures of less than 20°C. 160 kg spruce chips (dry weight basis) were steamed for about 40 seconds

suspension containing fungus (5 g per ton on a dry weight basis), unsterilized corn steep liquor (0.5% on a dry weight basis), and water was added to the mixer. The inoculated chips were rotated in V mixer for uniform mixing. These inoculated chips were dropped into a non-aerated silo (Figure 14). Six thermocouples were placed to monitor the temperature profile during the two-week period (Fig. 16). This silo was incubated in an incubator at 27°C for two weeks. This was done to avoid the effect of fluctuation of ambient temperatures on the biopulping performance of fungus under non-aerated conditions. After two weeks of fermentation, three samples of fungus-treated chips were collected; one sample from the location of thermocouple 2, second sample from the location of thermocouple 4, and third sample from the location of thermocouple 6. These samples were refined to measure the electrical energy consumption. Data presented in Table 11 show that sample 1 (thermocouple 2) saved 20% energy, sample 2 (thermocouple 4) saved 41% energy, and sample 3 (thermocouple 6) saved 35% energy. The average energy savings from this entire silo was about 32% which is very close to energy savings from laboratory scale biopulping experiments.

Table 11. Biopulping of *Ceriporiopsis subvermispora* on spruce chips in a silo reactor

Sample Identification	Energy savings over the untreated control (%)
Sample 1 (thermocouple 2)	20
Sample 2 (thermocouple 4)	41
Sample 3 (thermocouple 6)	35

These results from silo studies were also surprising to us because of the following unexpected

relationship between energy savings and temperature profile in the silo:

1. Fungus-treated chips (sample 1) collected from the location of thermocouple 2 saved 20% energy. However, this thermocouple 3-4 days out of 14 days after inoculation was exposed to more than 35°C (Figure 4). According to bioreactor studies, the fungus should not perform biopulping at or above 35°C (Table 6).

2. Fungus-treated chips (sample 6) collected from the location of thermocouple 6 saved only 35% energy. However, this thermocouple was exposed to less than 20°C for most of the time for two-week period (Figure 17). According to bioreactor studies, the fungus should not perform biopulping effectively and would have saved less than 18% energy (Table 1).

Conclusions, Examples 7 and 8

1. Fungus behaves very differently, but favorably on a larger scale.

2. In all of our previous inventions, forced aeration was used for effective biopulping. In this invention, we performed two silo experiments without any forced aeration. In both the experiments, 29-32% energy savings (average) were realized which are found to be comparable to those obtained with forced aeration both at a laboratory and 50-ton semi-commercial scales. These silo reactors are instrumented and insulated and represent an industrial size chip pile. No aeration would reduce the cost of biopulping operation substantially and would have a major impact on the economics of the process.

CLAIMS

1. A method for commercial scale biopulping of wood chips, said method comprising:

heating the surfaces of the wood chips to a temperature of at least about 90°C by applying steam to the surfaces thereof for a time sufficient to suppress the contaminating population of native, naturally occurring microbial flora, without complete sterilization of the wood chips;

cooling the wood chips to a temperature physiologically suitable for inoculation of a lignin-degrading and modifying species of white rot fungus;

inoculating the surfaces of the wood chips with an inoculum of the fungus suspended in a growth promoting nutrient adjuvant so that no substantial portion of the surfaces is uninoculated; and

incubating the wood chips under conditions of controlled temperature biocompatible with the propagation of the fungus until the lignin contained therein is degraded and modified to a desired extent.

2. A method for production of fungus inoculated wood chips in commercial scale, said method comprising:

feeding the wood chips continuously into a conveyor;

passing the wood chips in proximity to a source of a heat transfer medium;

heating the wood chips to a surface temperature of at least about 90°C by applying the heat transfer medium to the wood chip surfaces being exposed substantially uniformly to the steam by agitation during conveyance;

cooling the wood chips during conveyance to a temperature physiologically suitable for inoculating fungi;

passing the wood chips in proximity to a source of fungal inoculum;

inoculating the wood chips continuously by adsorbing the inoculum onto the wood chip surfaces being exposed uniformly to the inoculum by agitation during conveyance; and

collecting the wood chips so inoculated.

3. A method for commercial scale biopulping of a wood chip mass, said method comprising:

heating the surfaces of the wood chips to a temperature of at least about 90°C by applying steam uniformly to the surfaces thereof for a time sufficient to suppress the contaminating population of native, naturally occurring microbial flora, without complete sterilization of the wood chips;

cooling the wood chips to a temperature physiologically suitable for inoculation of a lignin-degrading and modifying species of white rot fungus;

inoculating the surfaces of the wood chips with an inoculum of the fungus suspended in a growth promoting adjuvant so that no substantial portion of the surfaces is uninoculated;

incubating the wood chips in sufficient wood chip mass that the inoculating fungus is conditioned to an elevation in temperature of the mass resulting from retention of heat generated by biological metabolism; and

aerating passively to provide a stream of oxygen to said wood chip mass.

4. The method of claim 3 wherein said wood chip mass is a chip pile or a mass of chips contained within a silo.

5. A method of treating wood chips for fungal inoculating and bioprocessing of the wood chips, said method comprising:

feeding the wood chips into a conveyor;

conveying the wood chips over a distance on the conveyor;

agitating the wood chips during said conveying step such that the wood chips are moved sufficiently to permit access to a substantial portion of the surfaces of the wood chips;

heating the wood chips during said conveying step by passing the wood chips through steam;

cooling the chips during said conveying step by ventilating the wood chips;

inoculating a substantial portion of the surfaces of the wood chips with an inoculant comprising an inoculating fungi and a nutrient adjuvant; and

collecting the wood chips so inoculated.

6. An apparatus for continuously treating wood chips for fungal inoculation and bioprocessing, said apparatus comprising:

a conveyor capable of conveying the wood chips and including a means for agitating the wood chips such that a substantial portion of the surfaces of the wood chips is exposed to treatment;

a decontaminator positioned adjacent to said conveyor and capable of dispersing decontaminant on the wood chips such that naturally occurring organisms are at least partially disabled; and

an inoculator positioned adjacent to said conveyor and capable of dispersing inoculum onto a substantial portion of the surfaces of the wood chips.

7. The apparatus of claim 4, wherein said decontaminator comprises:

a heater including a heat source and being capable of heating at least a portion of the surfaces of the wood chips in the conveyor; and

a cooler including a temperature regulator and being capable of cooling at least a portion of the wood chips in the conveyor.

8. The apparatus of claim 4, wherein said inoculator is mounted to said conveyor such that inoculant may be dispersed within the conveyor.

9. The apparatus of claim 5, wherein said heater is mounted to said conveyor for heat transfer to the wood chips travelling in the conveyor.

10. The apparatus of claim 5, wherein said cooler is mounted to said conveyor for cooling the wood chips travelling in the conveyor.

11. The apparatus of claim 4, further comprising a collector capable of receiving the wood chips expelled from said conveyor.

12. The apparatus of claim 4, wherein said conveyor comprises a first conveyor portion and a second conveyor portion, said apparatus for treating wood chips further comprising:

a surge bin positioned proximately to the end of the first conveyor portion and capable of holding wood chips expelled from said first conveyor portion and operatively connected to said second conveyor portion and capable of feeding wood chips to said second conveyor portion.

13. The apparatus of claim 8, wherein said inoculator is mounted to said second conveyor portion.

14. The apparatus of claim 8, wherein said decontaminator comprises a heater and a cooler, and wherein said heater is mounted to said first conveyor portion.

15. The apparatus of claim 8, wherein said decontaminator comprises a heater and a cooler, and wherein said cooler is mounted to said second conveyor portion.

16. The apparatus of claim 5, wherein said heater comprises a steam heater and said decontaminant is comprised of steam, said heater being capable of spraying said steam over a substantial portion of the surfaces of said wood chips.

17. The apparatus of claim 5, wherein said cooler is a ventilator.

18. The apparatus of claim 4, wherein said conveyor comprises a screw conveyor.

19. The apparatus of claim 14, wherein said screw conveyor comprises an auger mounted within a housing.

20. The apparatus of claim 4, wherein said conveyor includes an input side and an output side, and wherein said apparatus is movable over a distance such that wood chips expelled from the output side of the conveyor are spread over a distance.

21. The apparatus of claim 4, wherein said conveyor is rotatable about a point separated from the output side of the conveyor, such that wood chips expelled from the output side of the conveyor are spread over an arc.

22. The apparatus of claim 4, further comprising a ventilator operatively positioned next to said apparatus such that wood chips expelled from said apparatus may be positioned over said ventilator, said ventilator being capable of forcing a ventilating fluid through a wood chip pile.

23. A method for treating wood chips for fungal inoculation and bioprocessing, said method comprising:

collecting a mass of wood chips that have been inoculated with a fungal inoculum;

providing a ventilator capable of forcing a ventilating fluid through the mass of wood chips;

positioning the mass of wood chips over the ventilator in a configuration appropriate for incubation of the fungal inoculum; and

ventilating the mass of wood chips by forcing ventilating fluid through the mass of wood chips from the ventilator.

24. The method of claim 21, wherein the ventilating fluid is humidified air vapor.

25. The method of claim 21, wherein the ventilation rate is an aeration of about .02 to 0.7 volume/volume/minute for a mass of wood chips of greater than about 40 tons.

26. A regulated wood chip incubator, said incubator comprising:

a mass of wood chips, said mass of wood chips including a cover chip portion and an interior portion substantially within said cover chip portion; and

a ventilator including:

a plenum positioned within said interior portion of said mass and capable of exhausting a ventilating fluid into said interior portion of said mass of wood chips;

a ventilation system operatively connected to said plenum and being capable of forcing the ventilating fluid into said plenum; and

a control system for regulating the flow of the ventilating fluid to the mass of wood chips.

27. The regulated wood chip incubator of claim 24, wherein said control system includes a means for regulating the flow of the ventilating fluid in response to the temperature of said mass of wood chips.

28. The regulated wood chip incubator of claim 24, wherein said ventilating fluid is humidified air vapor.

29. The regulated wood chip incubator of claim 24, wherein the ventilation rate is an aeration of about 0.02 to 0.8 volume/volume/minute for a mass of wood chips of greater than about 40 tons.

30. The regulated wood chip incubator of claim 24, wherein the cover chip portion comprises uninoculated wood chips and the interior portion comprises inoculated wood chips.

31. A method for commercial scale biopulping of wood chips, said method comprising:

heating the surfaces of the wood chips to a temperature of at least about 90°C by applying steam to the surfaces thereof for a time sufficient to suppress the contaminating population of native, naturally occurring microbial flora, without complete sterilization of the wood chips;

cooling the wood chips to a temperature physiologically suitable for inoculation of a lignin-degrading and modifying species of white rot fungus;

inoculating the surfaces of the wood chips with an inoculum of the fungus suspended in a growth promoting nutrient adjuvant so that no substantial portion of the surfaces is uninoculated; and

incubating the wood chips in a pile having a surface area to volume ratio of about 2.5:1 to 6.5:1 so that the internal temperature of the pile reaches a temperature of about 35°C to 45°C in about two weeks.

32. A composition of matter for commercial scale biopulping comprising:

a pile of wood chips having a mass of greater than about 60 tons, the pile having a surface area to volume ratio of about 2.5:1 to 6.5:1, the wood chips inoculated with a lignin degrading fungi.

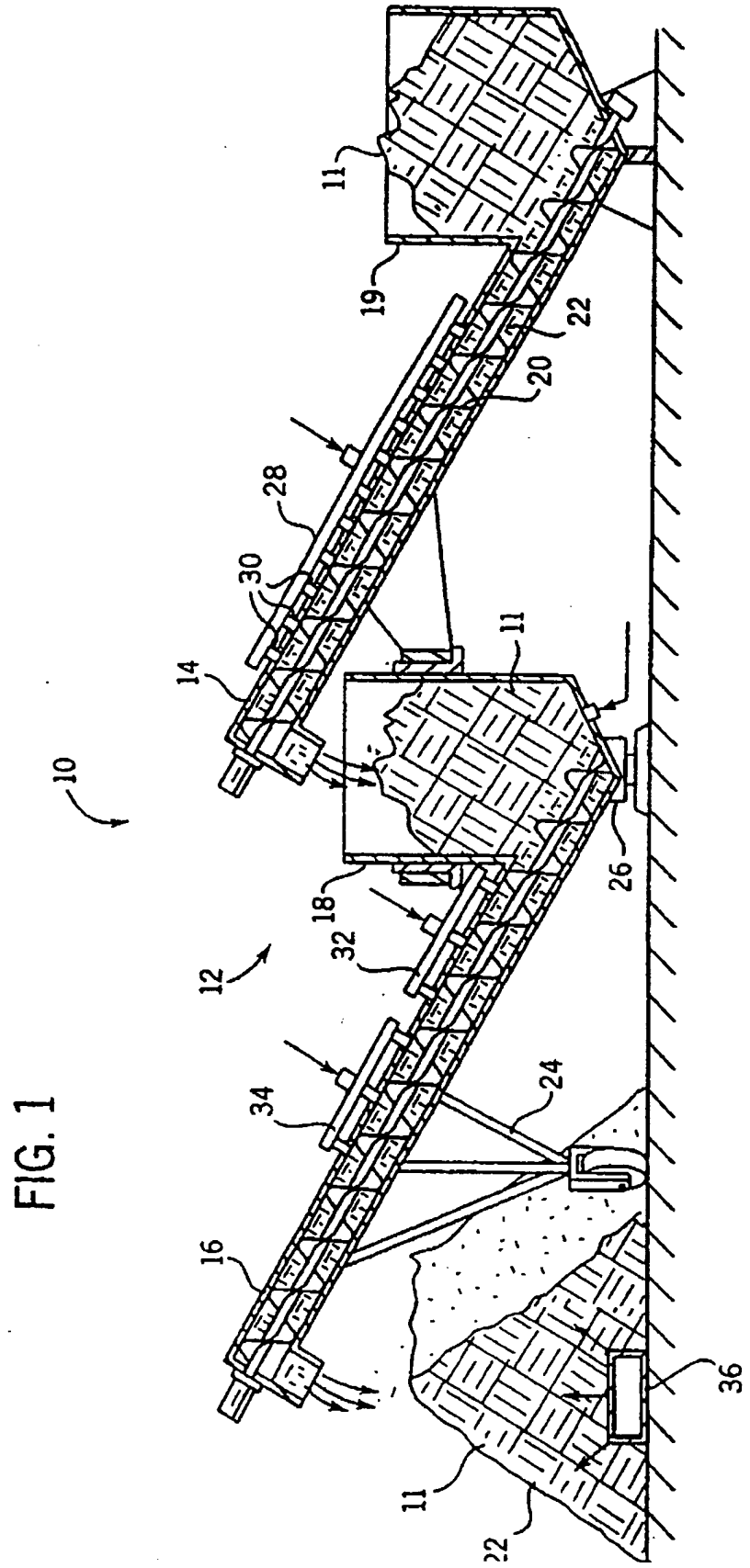


FIG. 1

FIG. 2

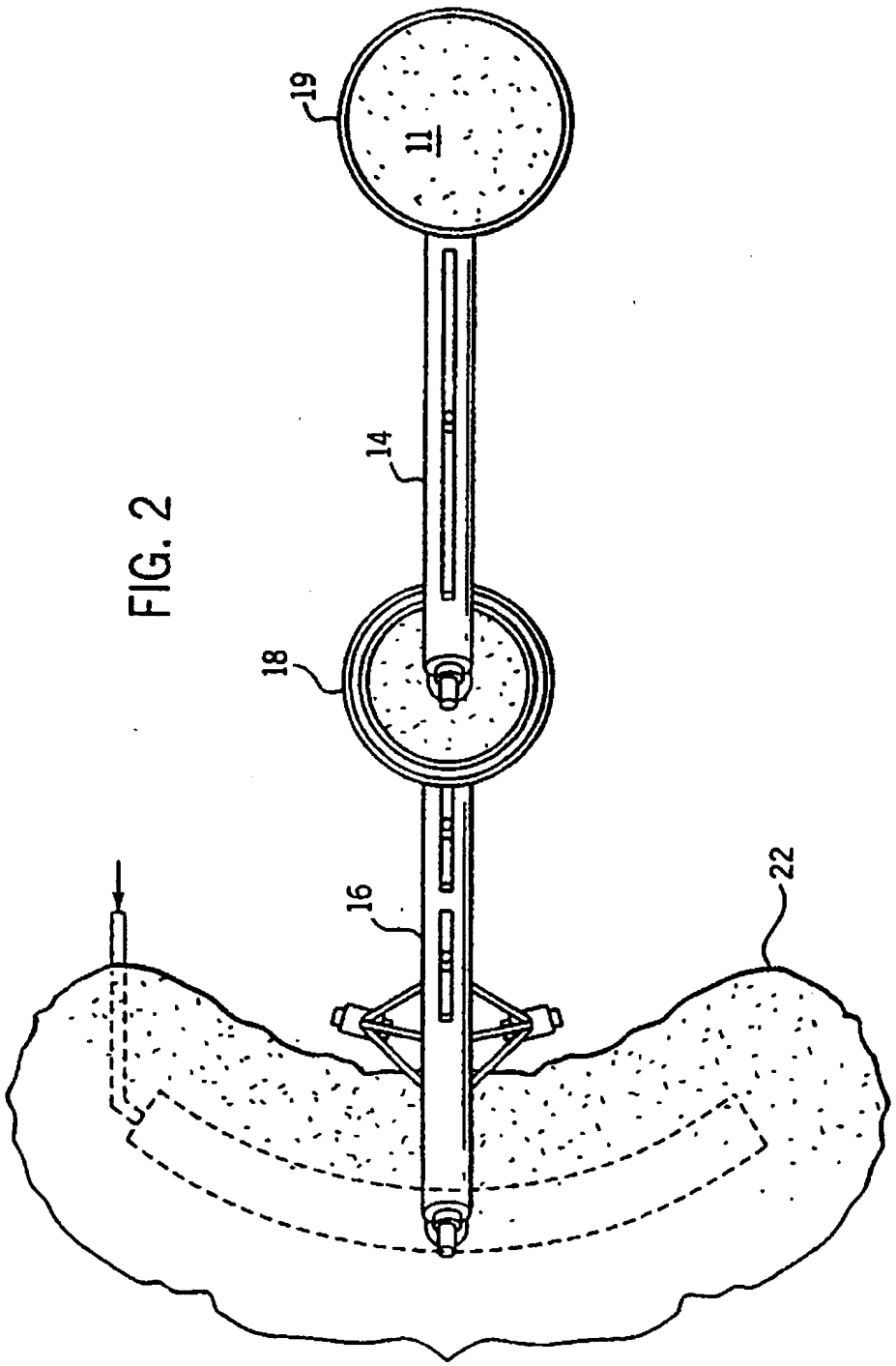


FIG. 3

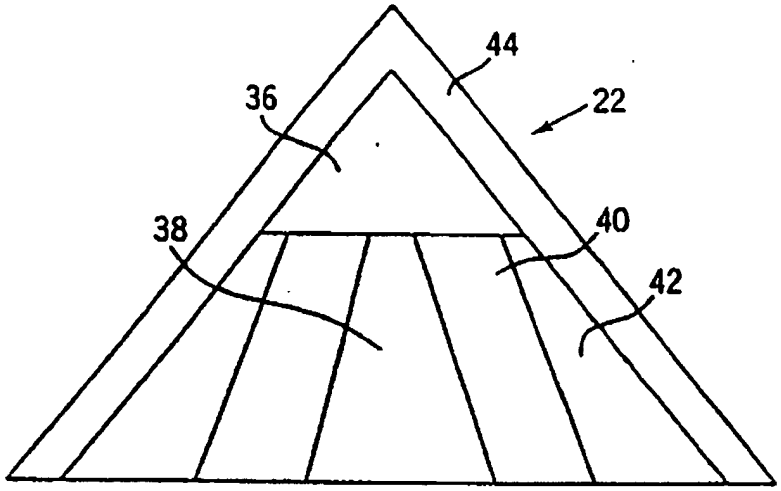


FIG. 4

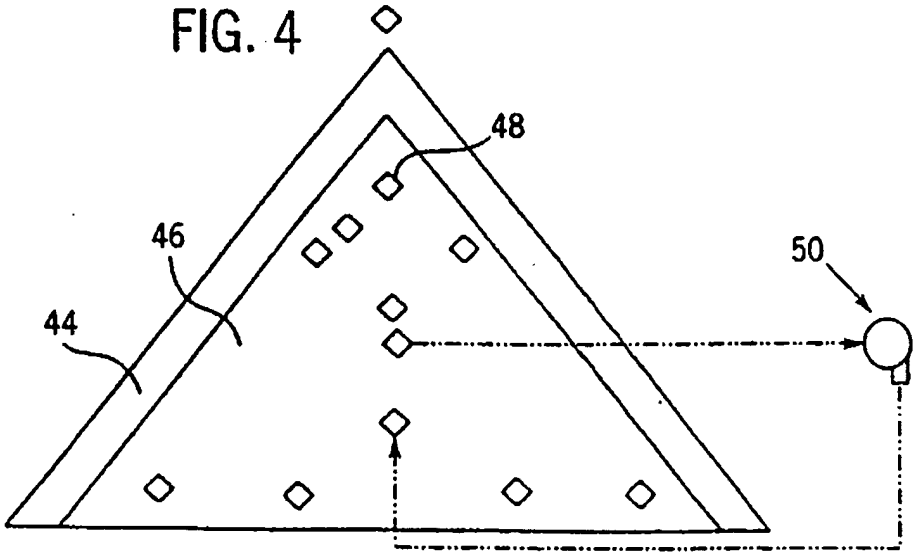


FIG. 5

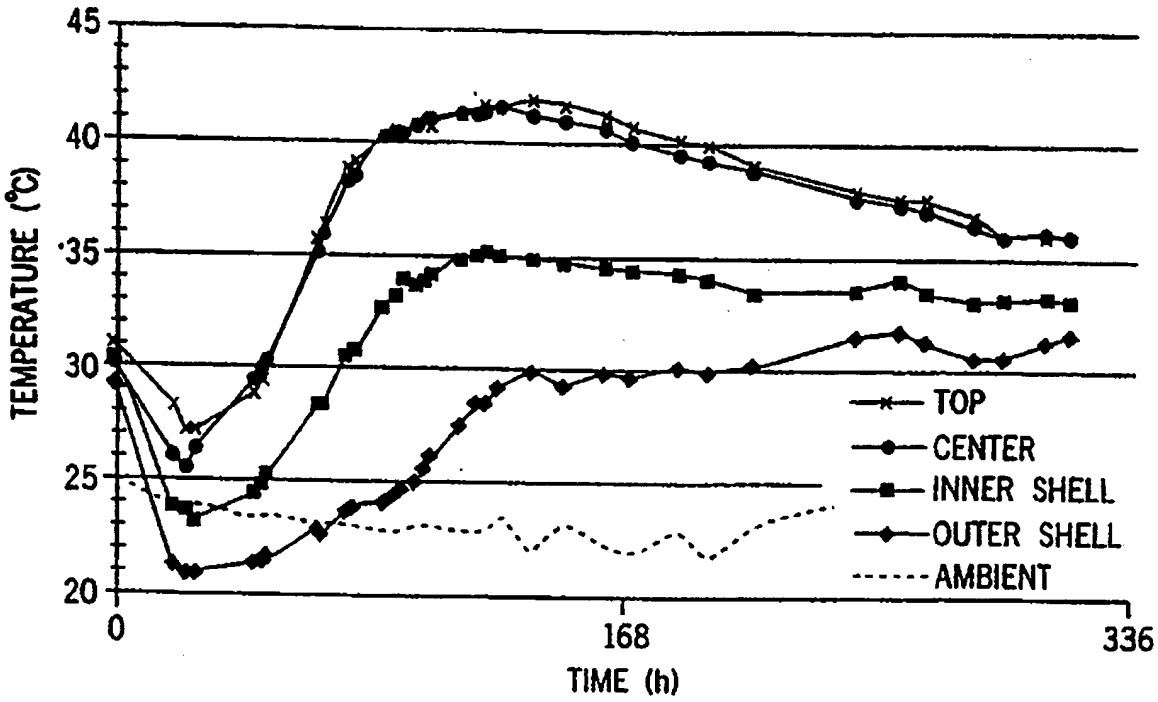

45
40
35
30
25
20
TEMPERATURE (°C)
0
168
336
TIME (h)
TOP
CENTER
INNER SHELL
OUTER SHELL
AMBIENT

FIG. 6

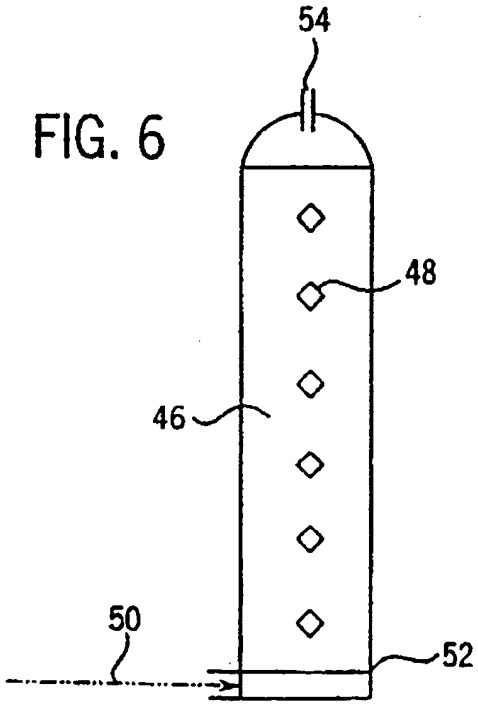

54
48
46
50
52

FIG. 7

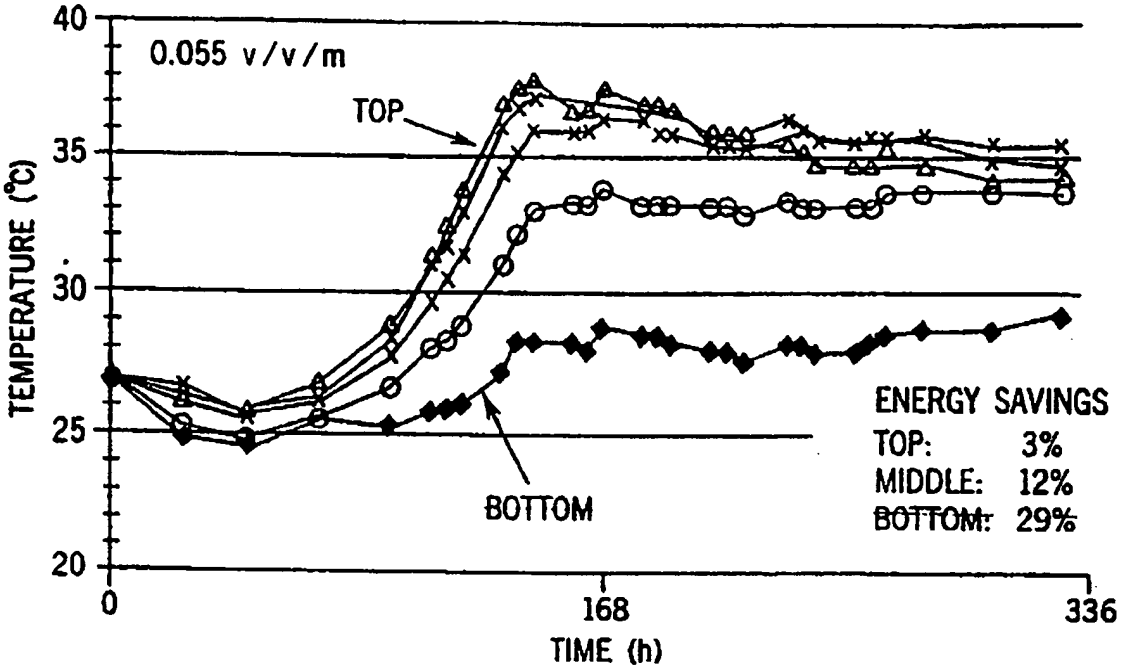

0.055 v/v/m
TOP
BOTTOM
TEMPERATURE (°C)
TIME (h)
40
35
30
25
20
0
168
336
ENERGY SAVINGS
TOP: 3%
MIDDLE: 12%
BOTTOM: 29%

FIG. 8

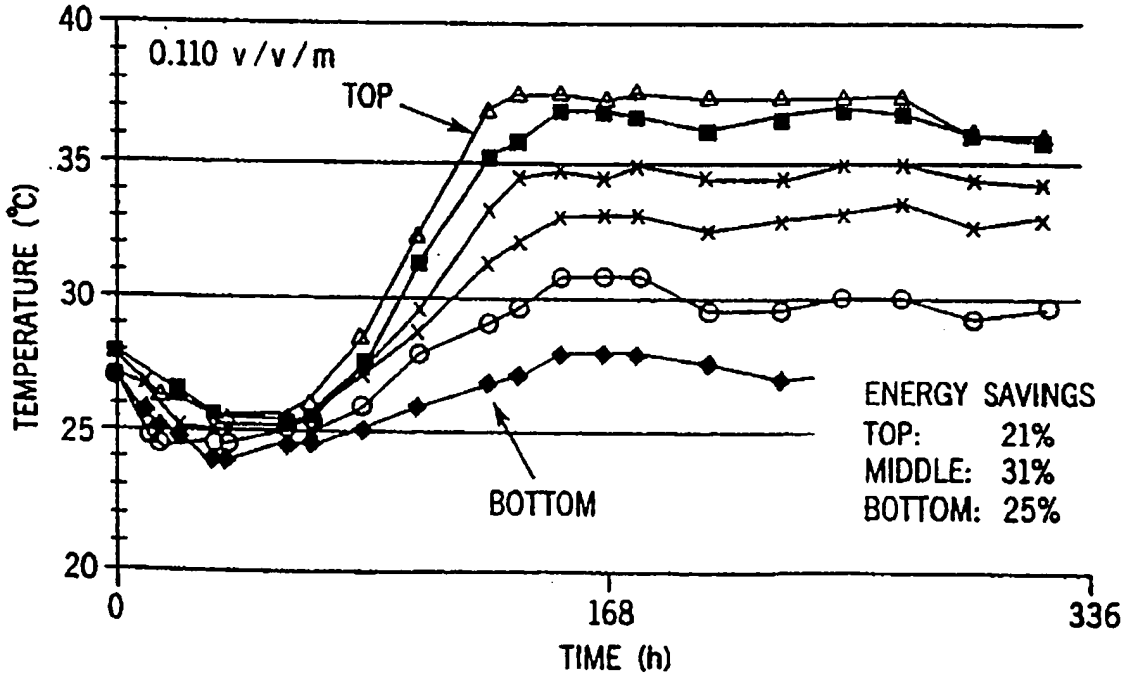

0.110 v/v/m
TOP
BOTTOM
TEMPERATURE (°C)
TIME (h)
40
35
30
25
20
0
168
336
ENERGY SAVINGS
TOP: 21%
MIDDLE: 31%
BOTTOM: 25%

FIG. 9

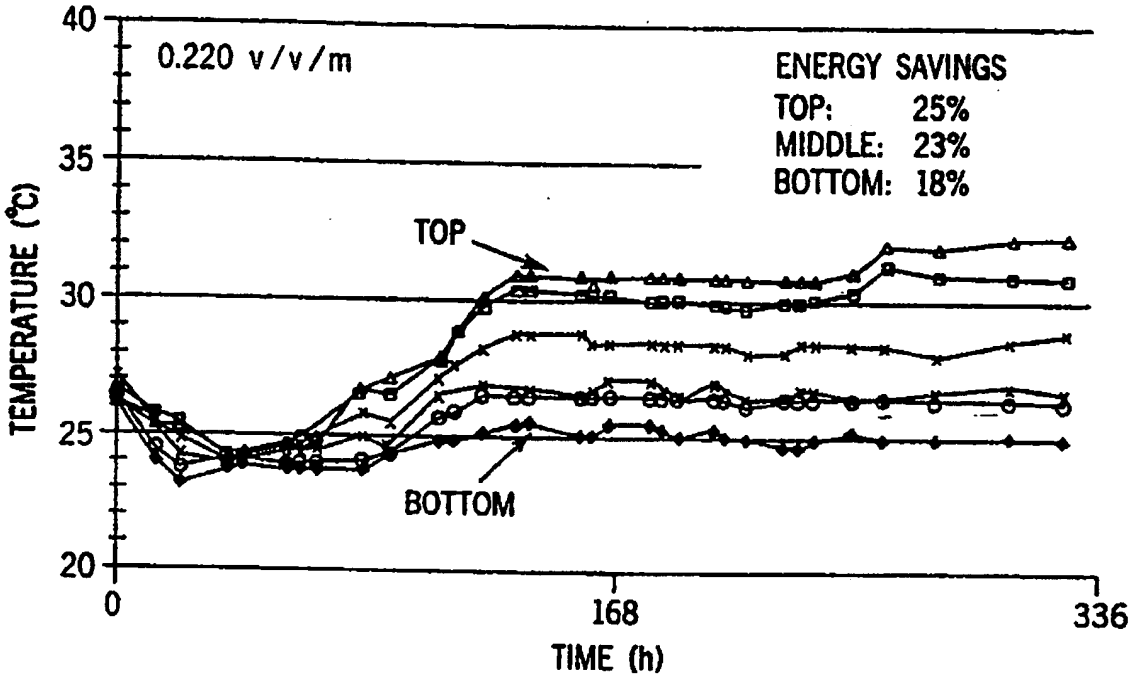

0.220 v/v/m
ENERGY SAVINGS
TOP: 25%
MIDDLE: 23%
BOTTOM: 18%
TOP
BOTTOM
TEMPERATURE (°C)
TIME (h)
40
35
30
25
20
0
168
336

FIG. 10

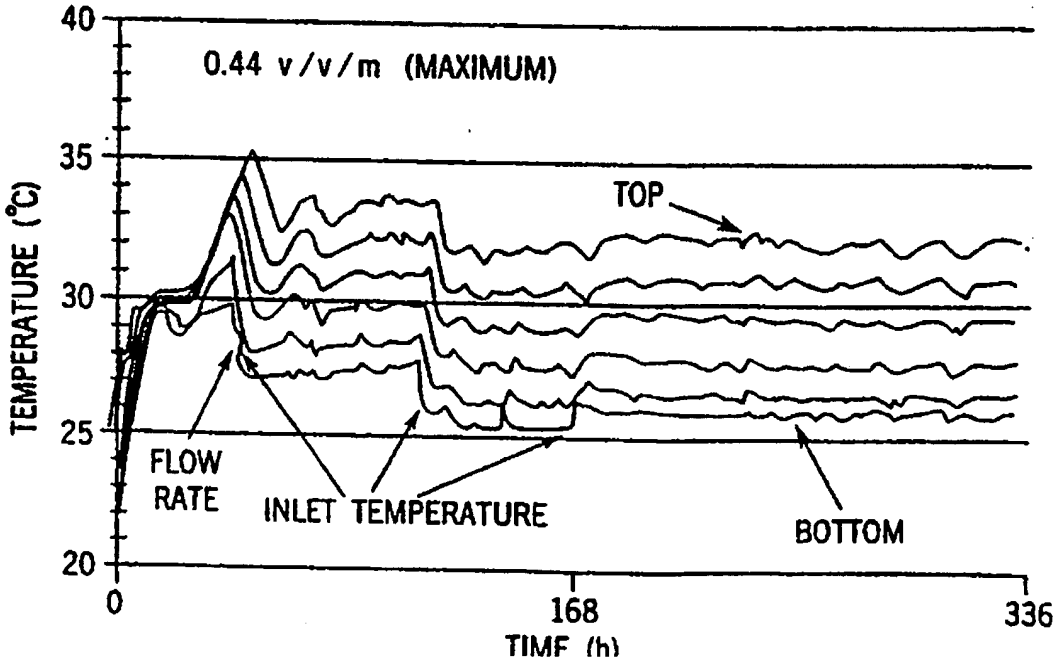

0.44 v/v/m (MAXIMUM)
TOP
FLOW RATE
INLET TEMPERATURE
BOTTOM
TEMPERATURE (°C)
TIME (h)
40
35
30
25
20
0
168
336

FIG. 11

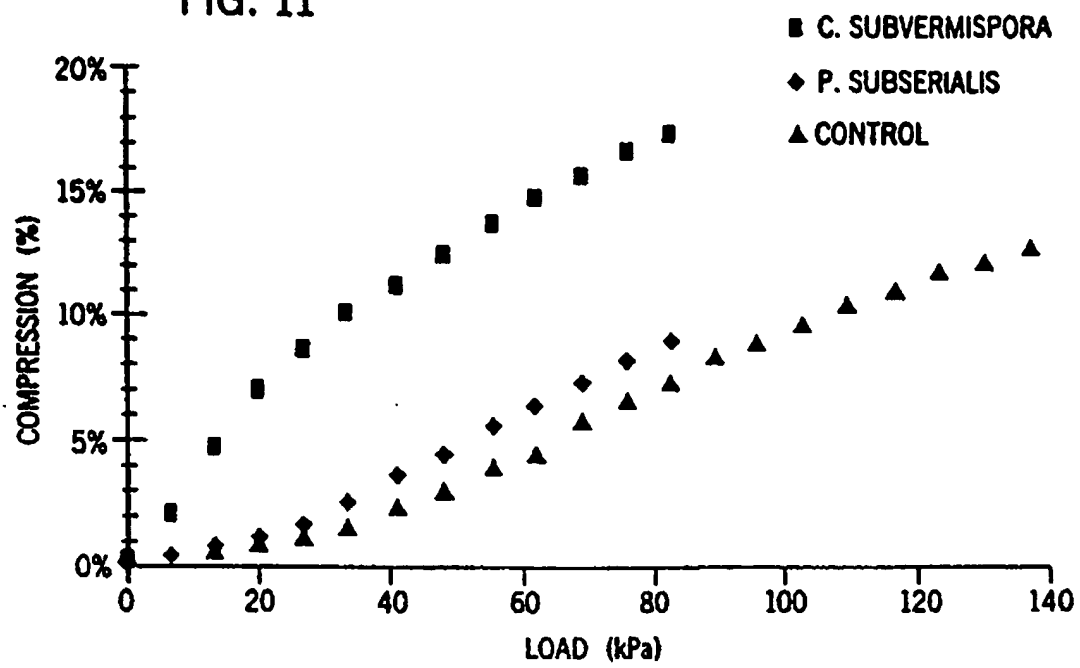

C. SUBVERMISPORA
P. SUBSERIALIS
CONTROL
COMPRESSION (%)
20%
15%
10%
5%
0%
0
20
40
60
80
100
120
140
LOAD (kPa)

FIG. 12

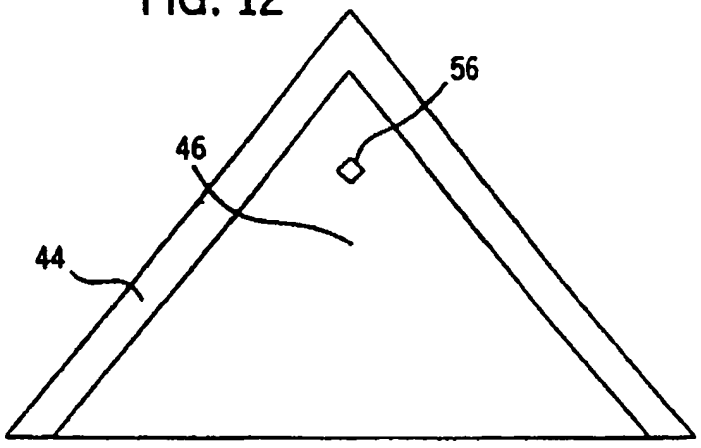
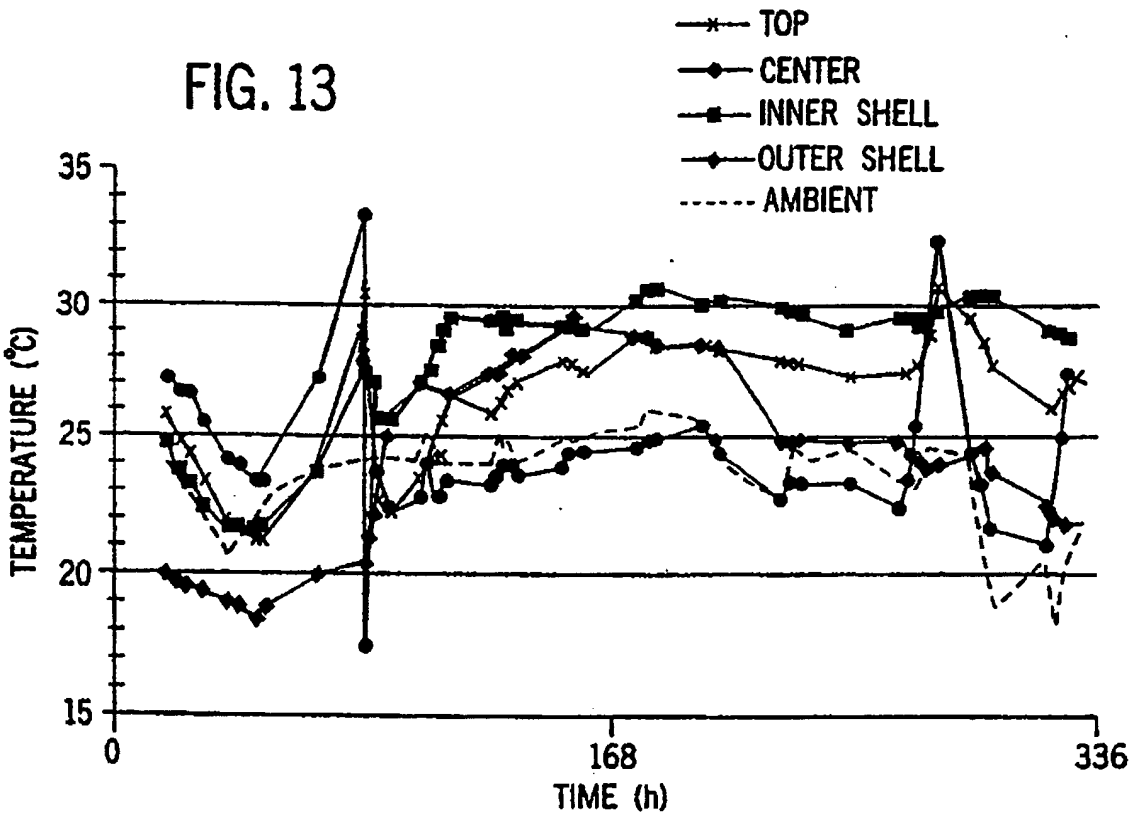

56
46
44

FIG. 13


TOP
CENTER
INNER SHELL
OUTER SHELL
AMBIENT
TEMPERATURE (°C)
35
30
25
20
15
0
168
336
TIME (h)

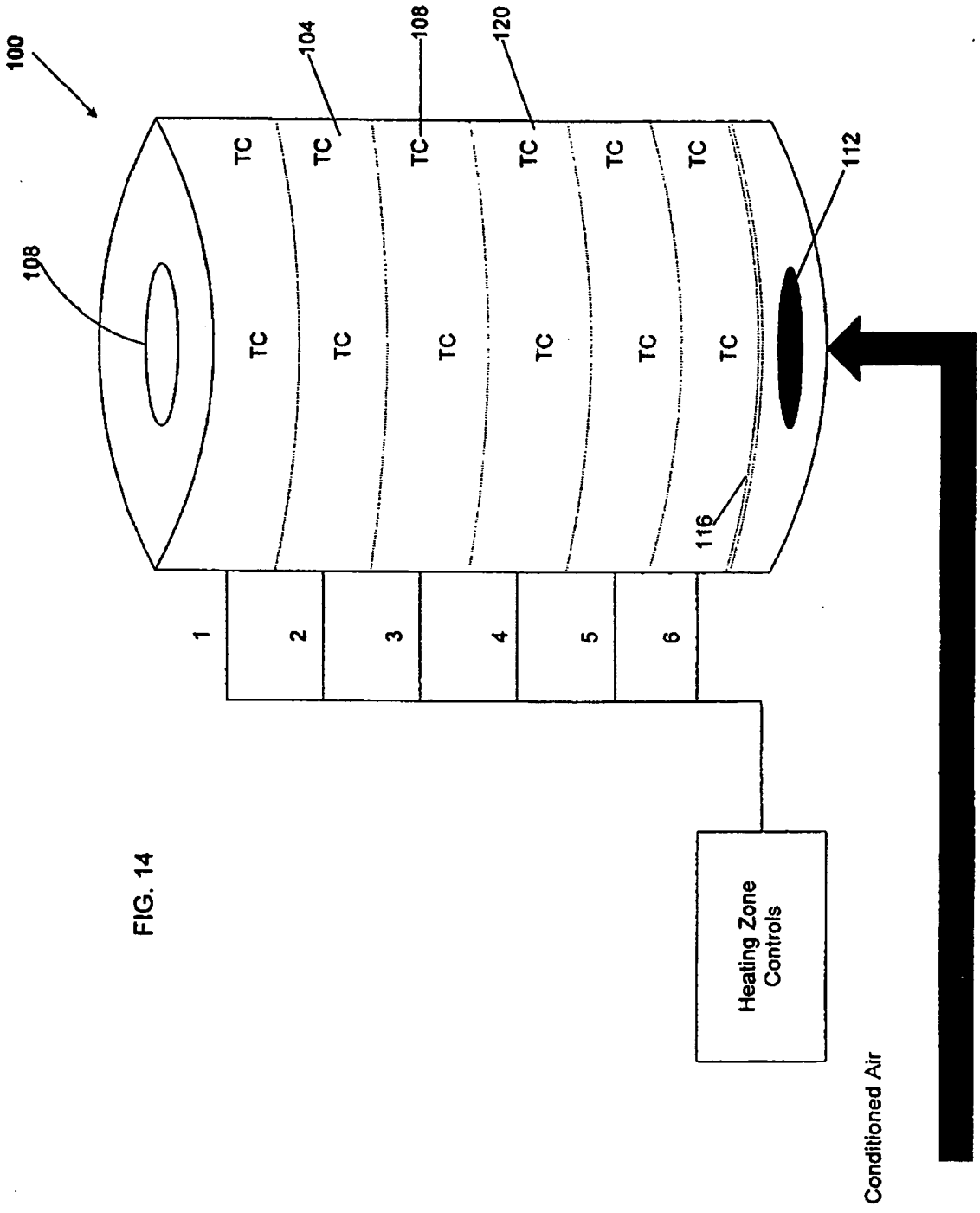

100
104
108
120
112
108
TC
116
1
2
3
4
5
6
Heating Zone Controls
Conditioned Air

FIG. 14

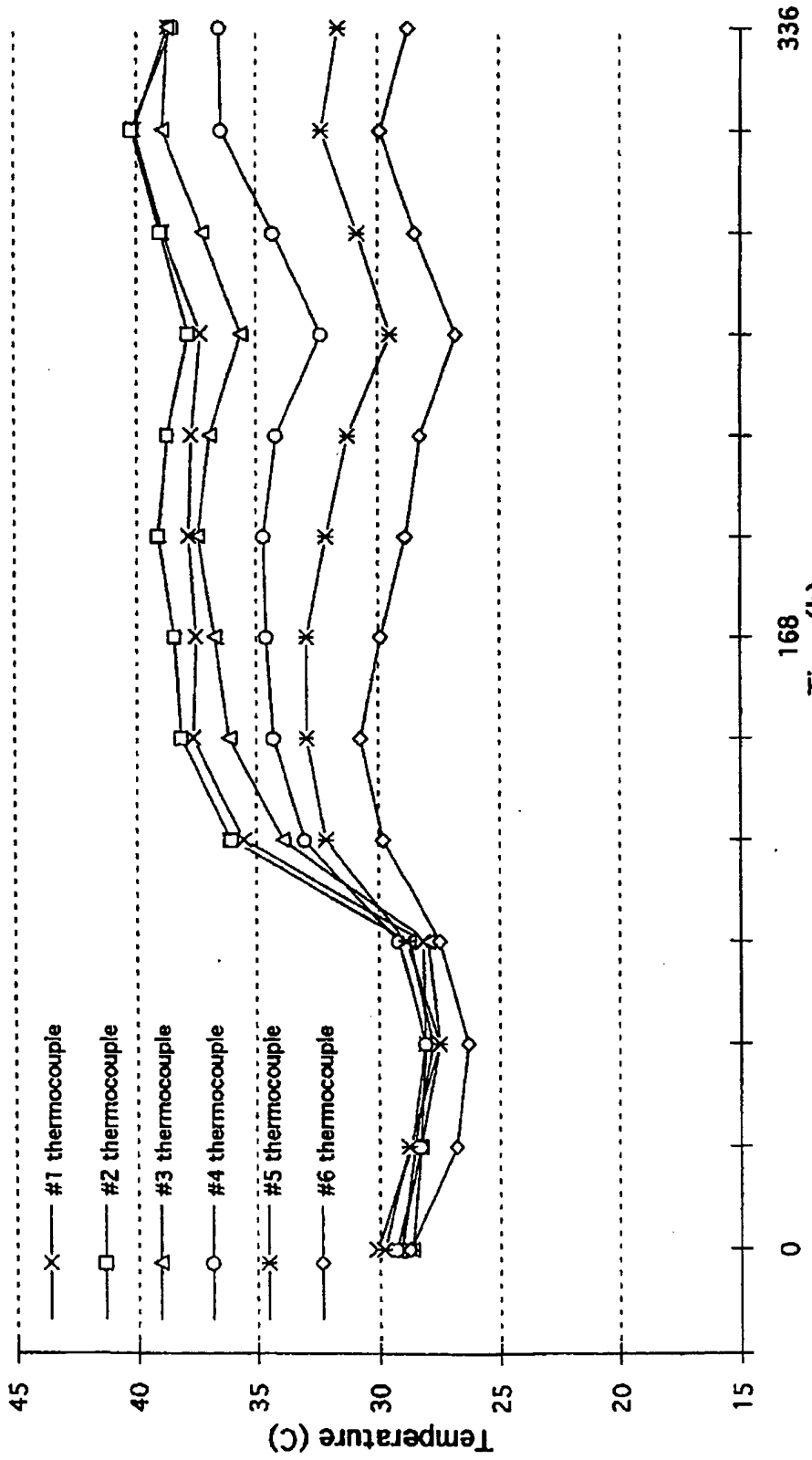

#1 thermocouple
#2 thermocouple
#3 thermocouple
#4 thermocouple
#5 thermocouple
#6 thermocouple
Temperature (C)
45
40
35
30
25
20
15
0
168
336
Time (h)

FIG. 15

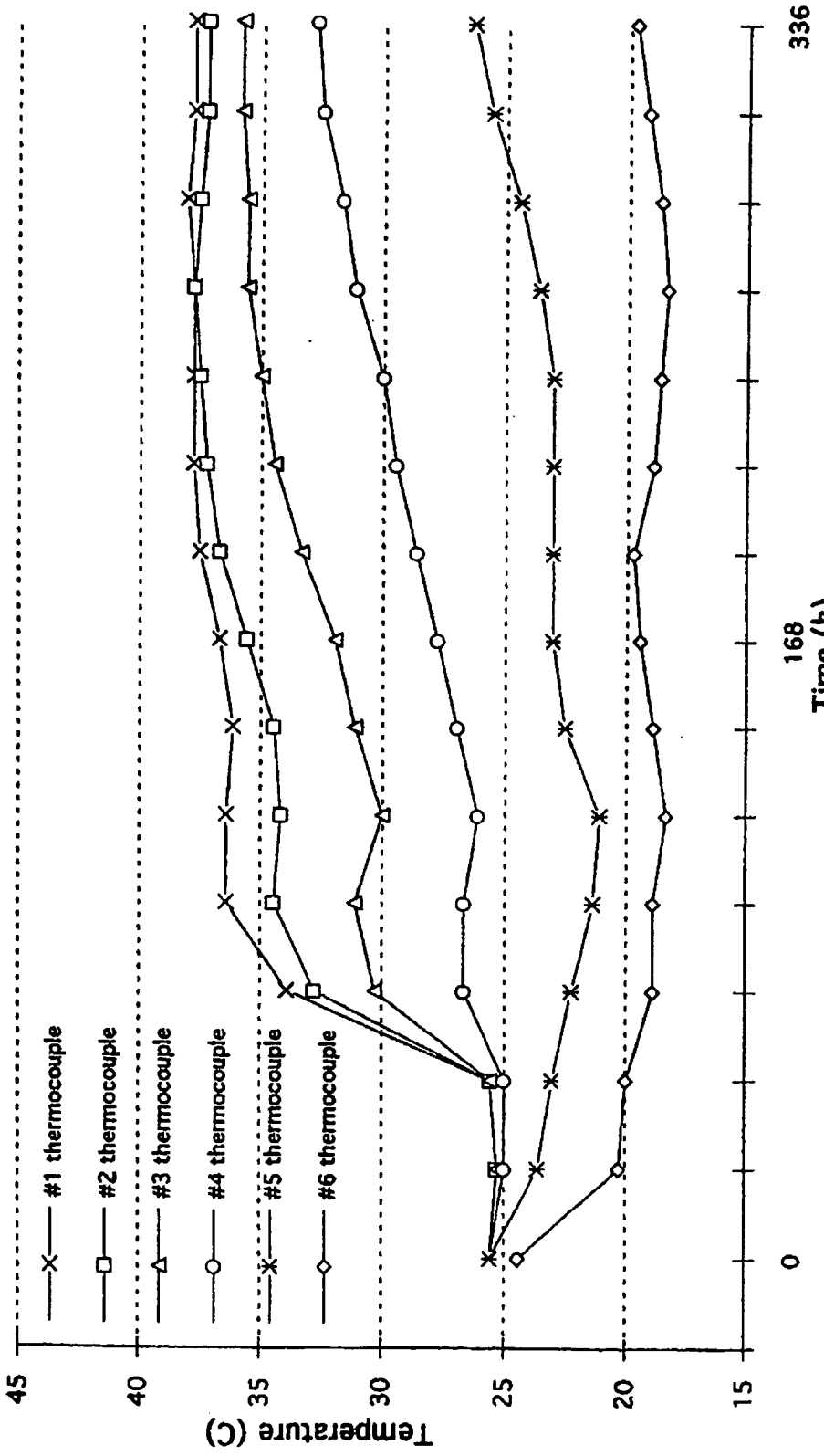

#1 thermocouple
#2 thermocouple
#3 thermocouple
#4 thermocouple
#5 thermocouple
#6 thermocouple
Temperature (C)
45
40
35
30
25
20
15
0
168
336
Time (h)

FIG. 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/05938

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :D21H 25/02
US CL :162/72, 237, 243, 246; 435/277, 278
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 162/72, 237, 243, 246, 250; 435/277, 278

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

None

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

None

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,460,697 A (AKHTAR et al) 24 October 1995, abstract and column 2, lines 39-47.	1-32
Y	US 5,055,159 A (BLANCHETTE et al) 08 October 1991, column 5, lines 32-39.	1-32
Y	US 4,088,529 A (RYHMAN et al) 09 May 1978, see Abstract.	1-32
Y	DILLNER, Forced Resin Seasoning of Sulfite Chips In a Silo, Annual Meeting of the Technical Association of the Norwegian Pulp & Paper INdustry, Oslo Norway, November 1976, see Figures 8 and 11.	2, 5-6, 8-15
Y	US 3,486,969 (NILSSON et al) 30 December 1969, see Abstract	2, 5-6, 8-15, 21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 JUNE 1998	Date of mailing of the international search report 07 JUL 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT	Authorized officer B. Hardy

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/05938

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2,991,281 (BRADSHAW et al) 04 July 1961, see Figure 1.	18, 19

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